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Functional and Structural Properties of Mammalian Acyl-CoA Thioesterases

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Abstract

Acyl-coenzyme A thioesterases (Acots) play important cellular roles in mammalian fatty acid metabolism through modulation of cellular concentrations of activated fatty acids. Acots catalyze the hydrolysis of the thioester bond present within acyl-CoA ester molecules to yield coenzyme A (CoASH) and the corresponding free fatty acid. Acyl-CoA thioesterases are expressed ubiquitously in both prokaryotes and eukaryotes, and in higher order organisms the enzymes are expressed and localized in a tissue-dependent manner within the cytosol, mitochondria, peroxisomes and microsomes. Recent studies have led to advances in the functional and structural characterization of many mammalian Acot family members. These include the structural determination of both type I and type II Acot family members, structural elucidation of the START domain of ACOT11, identification of roles in arachidonic acid and inflammatory prostaglandin production by Acot7, and inclusion of a 13th Acot family member. Here, we review and analyze the current literature on mammalian Acots with respect to their characterization and summarize the current knowledge on the structure, function and regulation of this enzyme family.
1.0 Introduction

Acyl-coenzyme A thioesterases (Acots) catalyse the hydrolysis of the thioester bonds of activated fatty acids to release the corresponding free fatty acid (FFA) and coenzyme A (CoASH). Substrates of these enzymes include a variety of fatty acyl ester molecules ranging from short- to long-chain, saturated and polyunsaturated fatty acyl-CoAs, branched-chain fatty acyl-CoAs and methyl-branched-chain fatty acyl-CoAs [1].

Acyl-CoA thioesterases are expressed ubiquitously by both eukaryotes and prokaryotes and have been isolated from a variety of organisms including bacteria, yeast, plants and animals. In higher order organisms the enzymes are localised to the cytosol, mitochondria, peroxisomes and microsomes. Within these compartments acyl-CoA thioesterases are distributed within a wide range of mammalian tissues and have been detected within brain, liver, kidney, heart, lung, steroidogenic and brown adipose tissues [2].

The Acots are broadly grouped into two classes (type-I or type-II) based on the molecular weight of the enzyme (see Table 1 for overview). There is no apparent sequence similarity between the two classes of Acots; however there is a high degree of sequence conservation between Acot family members within each class as shown in Table 2. The mouse genome contains six type-I Acot genes which are located within a cluster on mouse chromosome 12 D3 [3, 4]; in humans four type-I ACOTs are similarly located within a gene cluster on chromosome 14q24.3 [3, 4]. In contrast to type-I Acots, the genes encoding are type-II Acots are distributed throughout the genome.

A review of the nomenclature for acyl-CoA thioesterases was undertaken in 2005 by Hunt et al [4], and the suggested nomenclature has since been widely adopted; human enzymes are designated in upper case (eg ACOT1), while the mouse / rat homologues are designated in lower case (eg Acot1); the respective genes of each are denoted by italics (ACOT1, Acot1).
The wide cellular distribution and number of mammalian acyl-CoA thioesterases hampered early characterization efforts as individual enzymes were difficult to purify from tissue homogenates. Additionally, a loss of enzymatic activity following purification was commonly reported in the literature. It was not until the mid 1990s, when several cDNA clones encoding these enzymes were isolated, that individual Acot enzymes were able to be characterized.

2.0 Characterization of the mammalian acyl-CoA thioesterases

2.1 Subcellular localisation
Considering the putative roles of Acots in fatty acid metabolism, it is not surprising that considerable effort has been directed towards identifying the localisation of Acots within subcellular organelles such as mitochondria and peroxisomes. Sequence analysis of Acots has revealed both mitochondrial and peroxisomal targeting signals within many of the mammalian Acot family members.

Peroxisomal targeting signals (PTS) are mostly located at the C-termini of proteins [5, 6]. The importation of fully folded, even oligomeric, proteins into peroxisomes is mediated by the interaction of PTS with the peroxin family of import receptors [6]. Peroxisomal targeting signal 1 (PTS1) interacts with peroxin-5 (pex5p, PEX5) which was initially identified as a serine-lysine-leucine (–SKL) sequence, however variations of this prototypic consensus have now been identified [3, 5, 6]. PTS1 has been detected within the C-termini of several Acots; –SKL within Acot6 and Acot8 / ACOT8 [7-9], –PKL within human ACOT4 [3], and –AKL within mouse Acot3 and Acot5 [10]. However the putative PTS1 of ACOT1 (–SKV) and ACOT6 (–SKI) appear to mis-localise the protein to the cytosol [3].

In contrast to the PTS1 of peroxisomal proteins, mitochondrial targeting signals are located at the N-termini of proteins. Rat Acot2 (MTE-I) mRNA encodes a 42-amino acid N-terminal mitochondrial targeting signal, whilst the human homologue encodes a 62-amino acid N-terminal mitochondrial targeting signal [8].
2.2. Type-I acyl-CoA thioesterases: Acots 1-6

Acyl-CoA thioesterase 1 (ACOT1 / Acot1; also previously referred to as CTE-I, LACH2 and ACH2) [4] has been most extensively studied in rat liver [11-14]; however it has also been detected in rat brain [14], rat heart [14], rat kidney [14, 15] and rat testis [14]. An Acot1 homologue has also been reported in at least one plant species [16]. This cytosolic enzyme catalyses the hydrolysis of long-chain (C12 to C20) saturated fatty acyl CoA esters [8, 11, 12]. The enzyme also exerts activity, albeit decreased, against long-chain monounsaturated acyl-CoAs and methyl-branched-chain acyl-CoAs, and negligible activity against polyunsaturated fatty acyl-CoA esters [12]. The enzyme is inhibited by high levels of substrate [8]. The extensive characterization of Acot1 within rat liver has been possible due to an upregulation in the mRNA and protein expression of the enzyme, (which is normally only weakly expressed) through the administration of exogenous peroxisome proliferating agents [11, 13, 14]. Additionally, the expression of the enzyme can also be induced by fasting and diabetes [11].

Acyl-CoA thioesterase 2 (ACOT2 / Acot2; also previously referred to as MTE-I, PTE2 and ARTIS / p43) [4] is localised to the mitochondria within kidney, heart, liver, brain, brown adipose tissue (BAT), skeletal muscle and steroidogenic tissues [8, 15, 17, 18]. Rat Acot2 (MTE-I) mRNA encodes a 49.7 kDa protein [8], which includes a 45 kDa functional enzyme and a 42-amino acid N-terminal mitochondrial targeting signal, whilst the human homologue was initially thought to be a peroxisomal enzyme until its 62-amino acid N-terminal mitochondrial targeting signal was elucidated [8]. Stavinoha et al. [18] have postulated that Acot2 functions in concert with uncoupling protein 3 (UCP3) to increase fatty acid oxidation in rat cardiac and skeletal muscle [18]. The enzyme ARTIS (arachidonic acid-related thioesterase involved in steroidogenesis) was initially discovered within the zona fasciculata of the adrenal gland, and then subsequently within heart tissue [19], and was thought to be a novel thioesterase until molecular cloning demonstrated 100% sequence identity to MTE-I [8, 20]. The enzyme has also been detected in rat brain, mouse testis, human placenta and rat ovary suggesting an essential role in mammalian steroidogenesis [8, 20]. ACOT2 shares 93% amino acid sequence identity with ACOT1 [17], and therefore shares several functional characteristics. Most
notably, ACOT2 also acts on long-chain acyl CoA molecules (C_{14} to C_{20}) and is similarly upregulated in the rodent liver by the administration of peroxisome proliferators [8]. Cross-reactivity to monoclonal antibodies specific for ACOT1 is also commonly reported [15, 20].

Acyl-CoA thioesterase 3 (Acot3; PTE-Ia) is a peroxisomal enzyme that has only been characterized within the mouse [3]. Hunt and co-workers [3] demonstrated that the reduction in the number of peroxisomal Acots from four to two between the mouse and human genomes was a result of convergent evolution whereby human ACOT4 fulfilled the combined functions of mouse Acot3, Acot4 and Acot5 [3]. The enzyme, highly expressed in kidney, is upregulated following the administration of peroxisomal proliferating agents and by fasting, and catalyses the hydrolysis of long-chain acyl-CoA esters with the highest activity against palmitoyl-CoA (C_{16:0}) [10].

Acyl-CoA thioesterase 4 (ACOT4 / Acot4) is another peroxisomal thioesterase that is expressed mainly in kidney and to a lesser extent in liver and intestines. In mouse the enzyme exhibits a very high and specific activity towards succinyl-CoA, and to a lesser extent glutaryl-CoA, and then negligible activity against other short-, medium- and long-chain saturated and unsaturated fatty acyls [3, 21]. The human homologue also possesses high activity against succinyl-CoA, but then also exhibits a broad range of activity against short-chain dicarboxylic acids, and medium- to long-chain saturated and unsaturated monocarboxylic acyl-CoAs [3, 21]. Hunt and co-workers [3] postulate that this difference in specificity is the result of ACOT4 acquiring the combined functions of mouse Acot 3, 4, and 5 [3]. Long-chain dicarboxylic acids are chain-shortened within peroxisomes and then either excreted from the body in the urine or transported to mitochondria for further oxidation. Westin and co-workers [21] hypothesize that Acot4 might function to terminate the beta-oxidation of medium- and long-chain dicarboxylic acids through the removal of the CoA moiety, thereby producing the majority of succinate which is excreted in the urine [21].
Acyl-CoA thioesterase 5 (Acot5; PTE-Ic) is not present in human, where, as explained above, the equivalent function is undertaken by ACOT4. The mouse protein shares 82% amino acid sequence identity with mouse Acot3, and is expressed highest in spleen, brain, testis and intestine [10]. The activity of the enzyme differs from that of Acot3 and Acot4, with highest activity against medium-chain acyl-CoA esters, in particular decanoyl-CoA (C10:0) [10].

Acyl-CoA thioesterase 6 (Acot6) is a peroxisomal thioesterase that has been characterized as exhibiting a high specificity for the methyl-branched fatty acids phytanoyl-CoA (3,7,11,15-tetramethylhexadecanoyl-CoA) and pristanoyl-CoA (2,6,10,14-tetramethylpentadecanoyl-CoA) [7, 22]. The dietary precursor to these fatty acids is phytanic acid; the inability of the body to metabolise this fatty acid causes Refsum’s disease [22]. Although the mouse enzyme has been successfully cloned and characterized, attempts to recombinantly produce the full-length human enzyme have thus far been unsuccessful. An apparent full-length version of the enzyme is encoded by ACOT6, yet the corresponding mRNA was not detected in human tissue by Hunt et al. [3], and only a truncated version of the protein could only be cloned and expressed recombinantly [3].

A unique feature of the rodent type I Acots is their ability to be upregulated by peroxisome proliferating agents. Many laboratories have taken advantage of this feature in order to over-express the proteins, thereby allowing their characterization. The mechanism behind this feature is explored in Section 4.2.1.

All the type I Acots / ACOTs appear to be the result of gene duplications [3]. The open reading frames for all the enzymes are coded for by three exons; the exception is ACOT6 for which, as mentioned above, the full-length enzyme has not been detected nor recombinantly expressed [3]. Interestingly, a comparison of the genomic organisation of the mouse and human gene clusters shows a ‘switching’ of the positions of Acot1 / ACOT1 and Acot2 / ACOT2 between the two genomes [3]. Furthermore, the convergent
evolution of functions performed by ACOT4, as outlined above, suggests a level of functional redundancy in the mouse genome [3].

2.3. Type-II acyl-CoA thioesterases: Acots 7-13
Acyl-CoA thioesterase 7 (ACOT7 / Acot7; also formerly known as BACH, CTE-II, ACT, ACH1, MTE-II, LACH1) [4] has been the most extensively studied acyl-CoA thioesterase, and has gained attention in the literature in recent years for its specificity for arachidonoyl-CoA and purported role in inflammation. Seven isoforms of this type-II ACOT have been identified, which are a result of alternate splicing events in the ACOT7 gene [4, 23].

Acot7 was initially characterized as a rat liver enzyme with a broad range of activity towards medium- to long-chain acyl-CoAs (C8-C16) [14]. Immunoblotting using anti-Acot7 antibodies demonstrated the presence of the enzyme within rat brain and testis [14]. Molecular cloning confirmed previous reports that the enzyme was constitutively and highly expressed in rat brain and testis and induced in liver following peroxisome proliferator treatment [24]. The cloning of ACOT7 revealed the genomic organisation of the gene, leading to speculation that alternative splicing events within the first exon of the gene generated the various isoforms of the enzyme that had been previously detected [25]. Subsequently, in 2002 the mouse brain homologue of the enzyme was cloned, and sequence analyses demonstrated a greater than 95% amino acid identity to both the rat and human enzymes [26]. The rat homologue has been reported as being inhibited by adenosine triphosphate (ATP).

The emerging role of Acots in fatty acid metabolism led one group of researchers to seek out patients with suspected mitochondrial fatty acid oxidation disorders in an effort to identify individuals with specific acyl-CoA thioesterase deficiencies. Lowered thioesterase activity in the human skin fibroblasts of three patients corresponded to lowered levels of ACOT7 when analysed by western blotting, thus clinically linking acyl-CoA thioesterases to fatty acid metabolism [27]. Interestingly, it was demonstrated that approximately 90% of the thioesterase activity within the fibroblasts was attributed to
Further investigations demonstrated that whilst the major isoform of the enzyme (mouse Acot7a) was highly expressed within the brain and testis, the tissue-specific expression of the other isoforms (mouse Acot7b – 7e) was detected within heart, lung, spleen, kidney and liver [28]. Subsequent studies have also shown that mouse Acot7 is highly expressed in macrophages, and that gene expression can be further upregulated by factors that activate macrophages, including lipopolysaccharide (LPS) and colony-stimulating factor 1 (CSF-1) [29].

Acyl-CoA thioesterase 8 (ACOT8 / Acot8) possesses several distinguishing features. Firstly, this peroxisomal enzyme exhibits the broadest range of activity of all the Acots, with activity being reported toward short-, medium- and long-chain, branched-chain and methyl-branched-chain acyl-CoAs [8, 30]. In fact, this lack of substrate specificity led to the proposition that binding occurs through the CoASH moiety, and not the acyl moiety [30]. Competitive inhibition by free CoASH also supports this theory [30]. Secondly, the enzyme is reported to interact with and activate the HIV-1 Nef protein [8, 30]. The viral Nef protein is an essential protein for the pathogenesis of HIV-AIDS due to its ability to down-regulate the expression of several major histocompatibility (MHC) markers (particularly CD4) on T cells [30]. Significantly, Nef function is reportedly impaired if its interaction with ACOT8 is removed [30].

Two apparent isoforms of a murine 48-kDa polypeptide tentatively named p48 were characterized by Poupon et al. in 1999 [31]. Both isoforms produced mitochondrial thioesterase proteins containing 439 residues, sharing 95% nucleotide identity [31]. These Acots were characterized as being ubiquitously expressed due to their presence in a wide range of mouse tissues (including heart, brain, spleen, lung, liver, skeletal muscle, kidney and testis) [31]. The recombinant expression of the enzymes and spectrophotometric analyses demonstrated activity against medium- to long-chain acyl-CoAs, with the highest activity towards myristoyl-CoA (C14:0) [31]. Both proteins contain a 20-amino acid N-terminal mitochondrial targeting sequence, and this localisation was confirmed via immunofluorescence and confocal microscopy [31]. Genetic analyses identified that two genes were responsible for the translated proteins, and the enzymes were
subsequently renamed Acot9 (encoded by chromosome X) and Acot10 (encoded by chromosome 15) [4]. Homologues of Acot9 / ACOT9 have been identified in the proteome of several species (including frog, zebra fish, cow, pheasant and hydrozoan), whilst Acot10 has only been identified within the Mus genus.

Acyl-CoA thioesterase 11 (Acot11; also previously known as BFIT, BFIT1, BFIT2, Them1, KIAA0707) has been shown to be induced in mouse brown adipose tissue (BAT) by exposure to lowered temperatures, and repressed following exposure to warm temperatures [32]. Non-shivering thermogenesis in BAT is the main mechanism for maintaining body temperature upon exposure to a cold environment. Thermogenesis is mediated by the upregulation of uncoupling protein 1 (UCP1, thermogenin) that uncouples oxidative phosphorylation from the proton motive force. The result is that the energy from the oxidation of NADPH gets released as heat (to keep the organism warm) instead of electron flow and the generation of ATP. The upregulation of Acot11 as observed in mouse could therefore serve several purposes – it could cause an increase in FFAs available to release the inhibition placed upon UCP1, it could serve to decrease cellular levels of activated FAs thereby slowing beta-oxidation and futile ATP generation, and it could serve to regulate mitochondrial levels of CoASH [32]. Interestingly, obesity-resistant mice appear to have an approximately two-fold increase in transcription of Acot11 than compared to obesity-prone mice, leading the researchers to speculate on a potential link to obesity [32].

The molecular cloning, recombinant expression and functional characterization of mouse, rat and human ACOT12 / Acot12 have been reported [33-35]. All three cytosolic homologues exhibit high specificity toward acetyl-CoA, are activated by ATP and are inhibited by ADP [33-35]. Rat Acot12 has been postulated to be a biologically active homodimer (135 kDa) or homotetramer (240 kDa) [34]; however, based on the recently determined crystal structure, the quaternary structure of ACOT12 is likely to be a homotrimer (described in detail in section 5.2), similar to that of ACOT7 / Acot7.
Extensive spectrophotometric assays of human thioesterase superfamily member 2 (hTHEM2) has identified specificity towards a range of aromatic acyl-CoA including hydroxyphenylacetyl-CoAs and dihydroxyphenylacetyl-CoAs [36, 37]. Aromatic acyl-CoAs were assayed for activity based on a 20% sequence identity to the E. coli protein paaI which functions in the phenylacetic acid degradation pathway [37]. However, the lack of this pathway in humans led the researchers to conclude that these compounds are most likely not the physiological substrate [37, 38], and further studies suggest that perhaps medium- to long-chain fatty acyl-CoAs are the preferred physiological substrate with tight binding and low catalytic turnover actually being a mechanism of action [36]. Recent characterizations of the mouse homologue of this enzyme identified long-chain acyl-CoAs as the preferred substrate for the enzyme; subsequently these enzymes were re-assigned as Acot13 / ACOT13 [39].

3.0 Putative cellular functions of mammalian Acots

Acyl-CoA thioesterases are implicated in the regulation of many essential biological processes mediated through the modulation of the intracellular concentrations of activated FAs, FFAs and CoASH [8]. Apart from β-oxidation, activated fatty acids are involved in lipid biosynthesis, the allosteric regulation of enzymes, the regulation of ion channel opening, signal transduction, the budding and fusion of intracellular membranes and the regulation of gene transcription via nuclear receptors [8].

The requirement for free CoASH within mitochondria is very high, as it is a critical intermediate of the citric acid cycle, β-oxidation and other metabolic pathways; therefore, a readily available supply is essential for optimal mitochondrial function [8, 40]. The FFA products resulting from the cleavage catalysed reactions of Acots are also implicated in several essential biological processes. Arachidonic acid regulates calcium channels in cells, inducing calcium fluxes and a second messenger signalling cascade (for review see [41]). The preference of Acot7 / ACOT7 for arachidonoyl-CoA suggests that this enzyme could be an alternative source for the generation of arachidonic acid. Arachidonic acid also modulates voltage-gated ion channels, and it is the precursor molecule to eicosanoids (prostaglandins, leukotrienes and thromboxanes), which in turn are responsible for the
physiological manifestations of inflammation [42, 43], a critical immune response generated to counteract pathogenic infection.

3.1. Fatty acid metabolism and peroxisomal and mitochondrial acyl-CoA thioesterases
Beta-oxidation is the process through which fatty acids are degraded within peroxisomes and mitochondria to provide energy for essential cellular functions. Each compartment metabolises a specific set of activated fatty acids; peroxisomes metabolise very-long-chain and long-chain fatty acids, dicarboxylic acids, eicosanoids and bile acid intermediates, whilst mitochondria metabolise straight-chain saturated and unsaturated fatty acids [40, 44]. Within both organelles, fatty acyl esters are subjected to a series of enzymatic reactions to produce acetyl-CoA and a two carbon chain-shortened acyl-CoA [8, 40]. From peroxisomes, the resultant acetyl-CoA is transported to mitochondria where, along with the acetyl-CoA product, it either enters the citric acid cycle for further oxidation or is utilised in the production of ketone bodies to provide energy for extrahepatic tissues [8]. The chain-shortened products of peroxisomes are either excreted, or converted to carnitine esters and then also transported to the mitochondria for further oxidation [8, 30]. The observation that nine of the thirteen mouse Acots localise to mitochondria or peroxisomes strongly suggests an important role for Acots in fatty acid metabolism within these compartments.

One of the products resulting from β-oxidation of lipids within peroxisomes is propionyl-CoA (C3:0) [8, 30]. Propionyl-CoA is slowly oxidised and its accumulation within cells is reported to impair metabolism [8, 30]. The broad-range enzyme Acot8 has been identified as displaying activity toward propionyl-CoA within adipose tissue, and therefore may have a role in regulating levels of sequestered CoASH during times of elevated metabolic activity when high CoASH levels are required [8, 30]. Interestingly, this Acot undergoes substrate inhibition by CoASH, further supporting its role as a regulator of peroxisomal CoASH levels [12, 30].

Mitochondria possess the enzyme carnitine palmitoyl transferase II (CPT-II), which catalyses the conversion of carnitine esters (produced and routed from peroxisomes) back
to the acyl-CoA ester, so that it can then participate in mitochondrial β-oxidation [8]. During periods of fatty acid overload, when acyl-CoA ester levels are high, excess acyl-CoA esters are preferentially hydrolysed by Acots, rather than the reverse reaction, effectively reducing fatty acyl-CoA concentration [8]. Acetyl-CoA is an essential component in the citric acid cycle and in ketone production [8]. ACOT12 hydrolyses acetyl-CoA to acetate and CoASH [34]. This Acot is allosterically activated by ATP and inhibited by ADP, suggesting a role in the regulation of free CoASH concentration levels [34, 35].

3.2. The role of acyl-CoA thioesterase 7 in inflammation

Arachidonic acid (C20:4; AA) is released from membrane phospholipids by the action of phospholipase A2 (PLA2) [42, 45]. It can then be converted into either arachidonoyl-CoA (AA-CoA) by acyl-CoA synthetase (ACS) or into pro-inflammatory prostaglandins (PGs) by the cyclooxygenases (COX), or into the less inflammatory leukotrienes or anti-inflammatory lipoxins by the 5’lipoxygenase (5LO) pathway [43, 45]. Inhibition of ACS reportedly causes a substantial increase in PG levels, suggesting that ACS and COX compete against each other to utilize available AA [45].

Sakuma et al. demonstrated in 1994 that a cytosolic long-chain acyl-CoA thioesterase isolated from rabbit kidney medulla and with activity toward AA-CoA could be a mediator of cellular AA levels. The group proposed that a novel enzymatic pathway existed in which the cytosolic Acot was responsible for supplying free AA from the hydrolysis of AA-CoA, which could then be utilised in the synthesis of PGs [46]. Several years later the same laboratory further demonstrated that the microsomal fraction of rabbit kidney medulla also possessed this thioesterase activity, albeit to a lesser extent than the cytosolic fraction, which, using AA-CoA as a substrate, could regulate cellular levels of AA and therefore PG synthesis [47]. Coincidentally, a similar AA-releasing pathway involving a mitochondrial Acot (Acot2) in steroidogenic tissues was outlined in 2004 [48]. The cytosolic thioesterase is now known to be Acot7 / ACOT7. The cloning and characterization of the enzyme confirmed AA-CoA as the preferred substrate for Acot 7 / ACOT7, consistent with these earlier hypotheses [29]. Forwood et al. also
reported an upregulation in mouse *Acot7* mRNA transcription and an increase in thioesterase activity in mouse macrophages stimulated by lipopolysaccharide (LPS), conditions known to increase eicosanoid production [29].

### 3.3. The role of acyl-CoA thioesterase 7 in brain function

The expression of acyl-CoA thioesterases in the brain encouraged a number of groups to attempt to elucidate its specific role within this tissue. Following studies which identified the cellular and subcellular distribution of the enzyme, Yamada et al analysed the expression of the enzyme within the developing mouse brain and reported that the expression of *Acot7* increases from day twelve during embryogenesis until day seven following birth [49]. After this period, its expression declines until it reaches a level approximately 70% of the highest expression [49]. This expression occurred only within cells that had differentiated into the neuronal lineage, consistent with earlier studies that characterized Acot7 as being exclusively localised to neurons [26, 50].

There is an increase in neuronal cytosolic PLA$_2$ (cPLA$_2$) isoforms in neurodegenerative conditions such as Alzheimer’s disease, Parkinson’s disease, epilepsy and prion diseases; however, it is still yet to be determined whether this increase causes, or is a result of, the pathogenesis of the disease [51]. These conditions are also characterized by inflammatory reactions and oxidative stress, which can be attributed to increased AA levels brought about by the action of increased PLA$_2$ on membrane phospholipids [51]. The suggestion that Acot7 might also regulate brain function is strengthened by the linkage of the pathogenesis of mesial temporal lobe epilepsy in humans (MTLE) to the loss of ACOT7 within neurons in the hippocampus [52]. Two dimensional electrophoretic analysis identified ACOT7 as a protein absent from the hippocampus of MTLE patients [52], thus there is strong circumstantial evidence that ACOT7 is necessary for normal neuronal function and loss of function may result in neurodegeneration. Because free cytosolic long-chain fatty acyl-CoAs are cytotoxic (due to their detergent properties) and inhibit pyruvate kinase during glycolysis, one homeostatic role for Acot7 may be to limit the intracellular concentration of fatty acyl-CoAs below inhibitory and cytotoxic levels [49].
4.0 Regulation of mammalian acyl-CoA thioesterases

4.1 Regulation of acyl-CoA thioesterase activity by interaction with acyl-CoAs and coenzyme A

Acots play important cellular roles in lipid metabolism and it is therefore not surprising that their activity is regulated by molecules that signal the energy status of the cell. The identification of allosteric activators and inhibitors also provides useful functional insights. Substrate inhibition has been identified for Acot1 [12, 14], Acot3 [10] and ACOT8 [30], while enzymes such as Acot7 and ACOT8 have shown to be inhibited by high levels of free CoASH. In contrast, the type-I Acots Acot3, ACOT4, Acot5 and ACOT6, appear not to be sensitive to regulation [7, 10, 30]. The effect of FFAs upon the Acots has not been reported. Table 3 summarises endogenous and exogenous compounds that have been identified as modulating the behaviour of the acyl-CoA thioesterases.

4.2 Regulation of mammalian acyl-CoA thioesterase gene expression by transcription factors

4.2.1 Regulation of acyl-CoA thioesterases via peroxisome proliferator-activated receptors

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated transcription factors [53] of which three isotypes exist: alpha (PPARα), delta (PPARδ) and gamma (PPARγ) [42, 53]. The three PPARs, expressed by separate genes and displaying distinct tissue distributions, are implicated in the regulation of genes encoding enzymes that function in the metabolism of lipids, carbohydrates, bile acids and amino acids, and in inflammation [11, 42, 53]. PPARα mRNA is highly expressed in liver and has also been detected in heart, kidney, intestine, skeletal muscle and in several immune cells. Within hepatocytes PPARα regulates the genes responsible for the expression of enzymes involved in β-oxidation within peroxisomes, mitochondria, endoplasmic reticulum and the cytosol [11, 54]. PPARγ is highly expressed in adipose tissue and has also been detected in heart, kidney, intestine and spleen [54]. PPARδ is highly expressed in colon, small intestine, liver and keratinocytes [55].

Upon ligand binding to the C-terminal ligand-binding domain, PPAR associates with the retinoid X receptor (RXR) to form a heterodimer, which then binds to peroxisome proliferator response elements (PPREs) within the promoter / intron region of many
genes [11, 54]. Many endogenous molecules and exogenous compounds have been characterized as PPAR ligands [54]. Endogenous ligands for PPARα and PPARγ include FFAs, activated fatty acids and eicosanoids [10, 11, 54], with each isoform displaying specificity for different fatty acyl chain lengths and esterified or unesterified forms [56]. For example, PPARα exhibits a greater affinity for the esterified forms of very-long-chain and branched-chain fatty acids than for FFAs [56].

The exogenous compounds that activate PPAR are referred to as peroxisome proliferators (PPs). PPs are a diverse group of synthetic compounds such as Wy-14,643, plasticisers and hypolipidemic drugs that cause a pronounced proliferation of peroxisomes and hepatocytes within rodent liver due to their agonistic activation of PPARα [10, 13, 30, 54]. Neuman et al. demonstrated an increase in liver Acot2 mRNA transcription in rodents administered with di(2-ethylhexyl) phthalate (DEHP) [17]. The discovery that these compounds cause an upregulation in rat liver type-I Acot expression enabled some of the pioneering Acot characterisations, even though at the time the role of PPARα in the generation of this response was yet to be elucidated. However, surprisingly, this upregulatory effect is yet to be observed in humans [2, 54].

4.2.2. The regulation of acyl-CoA thioesterases via hepatic nuclear factor 4α
Hepatic nuclear factor 4α (HNF4α), expressed in liver, kidney, intestine and pancreas, is another transcription factor that regulates genes involved in hepatocyte differentiation, lipid and carbohydrate metabolism and haematopoiesis [11, 57]. Transcriptional activation occurs when homodimeric HNF4α binds to the direct repeat (DR-1) promoter sequences of target genes [11, 57]. Ligands of HNF4α include acyl-CoA ester molecules and natural and xenobiotic fatty acids; however the chain length and degree of saturation determines whether the transcriptional activity of HNF4α is activated or suppressed [11, 57].

Dongol and co-workers investigated the upregulation of Acot1 by peroxisome proliferators and found this effect to be regulated in a PPARα-dependent manner [11]. The (DR1) in the distal promoter region of mouse Acot1 that binds PPARα was identified
and found to also bind activated HNF4α, suggesting that activated PPARα and HNF4α might compete against each for binding to DR1 [11]. Considering that the activity of both PPARα and HNF4α can be modulated by fatty acids and fatty acyl-CoAs, it is postulated that the upregulation of Acot1 is due to a complex interplay based upon ligand availability and repressor / activator interaction [11].

4.2.3. The regulation of acyl-CoA thioesterases via sterol regulatory element-binding protein-2
Sterol regulatory element-binding protein-2 (SREBP-2) is a transcription factor that regulates genes involved in fatty acid and cholesterol synthesis [58]. When cellular cholesterol levels fall, proteolytic cleavage of the N-terminal domain of the membrane-bound SREBP occurs, allowing a nuclear form of SREBP-2 to translocate to the nucleus where it binds to the sterol regulatory element (SRE) within the promoter region of target genes [58]. Takagi and co-workers demonstrated that within neurons Acot7 was upregulated due to the binding of SREBP-2 to the SRE within the promoter region [58].

5.0 Structural characterisation of mammalian acyl-CoA thioesterases
Mammalian Acots are classified into two classes based on their molecular weight (see Table 1 for overview). While there is no apparent sequence similarity between type I and II Acots, there is a high degree of both sequence similarity and domain organisation within each class. A schematic summary of the domain organisation within the two Acot classes is presented in Figure 1.

5.1 Structures of type I Acots
All type I Acots contain an N-terminal β-sandwich domain and a C-terminal α/β hydrolase catalytic domain (Fig 1). The high degree of sequence conservation within this subfamily provides a strong basis on which to model the other type I Acots to infer structure / function relationships. The determination of the structure of ACOT2 (PDB ID: 3HLK) [59] has provided important insights into the active site and interactions between the β-sandwich and C-terminal α/β hydrolase catalytic domains. The N-terminal domain of ACOT2 comprises a seven-stranded β-sandwich. This β-sandwich domain contains one sheet made up of three short β-strands, and another sheet containing four longer
strands (Figure 2). While the N-terminal domain does not contribute catalytic active site residues (these are within the C-terminal α/β hydrolase domain), the long loop connecting β-stands 4 and 5 contributes to the overall architecture of the active site. It is therefore possible that the N-terminal domain plays some role in modulating the specificity for acyl-CoA substrates, although this has not been formally tested.

The C-terminal domain of type I Acots has the α/β hydrolase fold, common to a wide range of hydrolytic enzymes as well as proteins with no recognized catalytic activity. In ACOT2 this domain consists of a centrally positioned 8-stranded β-sheet, surrounded by five α-helices (Figure 2). As with most family members within the α/β hydrolase family, the β-strands are parallel, with the exception of an inverted first strand showing antiparallel orientation. The catalytic triad within the domain is composed of Ser294, Asp388, and His422, all of which are located on the loops connecting the α-helices and β-strands.

5.2 Structures of type II Acots
Type II Acots (7-13) contain one or more copies of the hotdog fold domain. As shown in Figure 1, Acots7-10 share a tandem hotdog fold domain organisation, while Acots 11 and 12 possess an additional C-terminal START domain. Acot13, recently characterized as a type II acyl-CoA thioesterase based on its specificity for long-chain acyl-CoA substrates and likelihood to form a homotetramer in solution, possesses a single hotdog domain [39].

5.2.1 Structure of Acot7
The structure of Acot7 was determined in 2007 [29]. Recombinant N- and C-terminal hotdog domains were crystallised independently, and the juxtaposition of these domains in the full-length structure was determined using distance constraints determined by chemical crosslinking and mass spectrometry [60, 61]. In the quaternary structure, each tandem hotdog domain formed an intramolecular dimer, which then further associated into a trimer as depicted in figure 3. Each hotdog fold featured a 5-stranded antiparallel β-sheet “bun” enveloping a five-turn α-helix “sausage” with an additional C-terminal α-helix packed on the opposite side of the β-sheet (Figure 3) [62]. The hotdog fold, initially
described in FabA from *E. coli* is also commonly referred to as a 4HBT (4-hydroxybenzoyl-CoA thioesterase) domain in reference to the archetypal *Pseudomonas* enzyme from which the term “hotdog fold” was coined [62]. This fold has subsequently been identified within the structures of many prokaryotic and eukaryotic species [62].

Intriguingly, the individual hotdog domains of Acot7 also associated into the dimer-of-trimers configuration, however these individual domains were inactive across a broad range of substrates [29]. Activity could only be restored through combining the N- and C-terminal domains. Although not formally tested, given the sequence conservation, it is likely that the other tandem hotdog domains within the type II Acots are similar in this regard.

The low sequence similarity between the two fused hotdog domains means that the enzyme contains two spatially analogous, non-identical active sites, each repeated three times on opposing faces of the enzyme. Mutagenesis of Acot7 revealed that only one site was active; mutations within active site I (residues N24A and D213A) abolished activity, while mutations to the corresponding residues in active site II (residues E39A and T198A) did not affect activity [29].

Interestingly, bacterial homologues and other type II Acots also share this half-of-sites reactivity. For example, the single prokaryotic thioesterase domain of PaaI contains six identical active sites and its overall quaternary arrangement is similar to that of Acot7, and utilises only half of the active sites through an induced-fit mechanism of negative regulation [63]. It is also likely that ACOT12 possesses a similar half-of-sites reactivity based on the recently determined crystallographic structure (described below). It is proposed that this negative control is a general feature of all type II Acots and may be important for the ability of this enzyme family to modulate cellular concentrations of fatty acyl substrates and products.
5.2.2 Structure of ACOT11 START domain
Acots 11 and 12 each contain two tandem copies of the hotdog fold domain and a C-terminal START (steroidogenic acute regulatory transfer) domain. START domains are approximately 200 amino acids in length and bind various lipids to mediate intracellular functions including intracellular lipid transport, lipid metabolism, and cell signaling events. The human and mouse genomes each encode 15 START domain-containing proteins.

The x-ray crystallographic structure of the START domain of ACOT11 was deposited in the PDB in 2009 (PDB ID: 3FO5), however no journal publication describing the structure has been published. Here we present a preliminary analysis of the structural data. The overall structure contains an antiparallel β-sheet consisting of nine strands surrounded by five α-helices. A tunnel, formed by the β-sheet and α-helices 3, 4 and 5, surrounds a single pentaethylene glycol molecule (C₁₀H₂₂O₆), which based on structural similarity with other START domains that contain ligands such as dilinoleoylphosphatidylcholine (PDB ID: 1LN2), is likely to be the lipid binding site (Figure 4).

5.2.3 Structure of ACOT12
The structure of mammalian ACOT12 was deposited in the PDB in 2008 by the Structural Genomics Consortium (PDB ID: 3B7K). A detailed analysis of the structure has not yet been published; therefore for the purpose of the review we have briefly analyzed the structure with respect to other Acots.

ACOT12 exhibits the same hexameric arrangement of hotdog domains as that observed in the quaternary structure of ACOT7 / Acot7 (Figure 5), despite the low sequence identity between the two proteins (Acot7 and Acot12 sequence identity 24.9%). The tandem N- and C-terminal hotdog domains associate to form a double hotdog fold, three of which associate in a trimer.

The active site architecture appears similar to that of ACOT7 / Acot7. Of the two structurally analogous active sites, it is likely that only one is active based on
comparisons with Acot7 and the presence of coenzyme A molecules on only one face of the molecule (Figure 5). Thus, the half-of-sites reactivity described in Acot7, and which appears to be conserved in some prokaryotic thioesterases, is likely to be present in Acot12.

6.0 Conclusion
There have been many significant advances in recent years with respect to the functional and structural characterization of both type I and II mammalian Acots. Elucidation of functional roles revealed the association with lipid biosynthesis, allosteric regulation of enzymes, regulation of ion channel opening, signal transduction, budding and fusion of intracellular membranes, and the regulation of gene transcription via nuclear receptors. There is also evidence to suggest that Acots may be linked to neurodegenerative diseases, epilepsy, obesity and inflammation.

Significant inroads have also been made with respect to the regulation of Acot function. Activity has been shown to be regulated by ATP, coenzyme A, free and activated fatty-acids, while expression of the Acot genes has been shown to be regulated by peroxisome proliferator-activated receptors and hepatic nuclear factor 4α. Half-of-sites reactivity is likely to be a common feature of type II Acots; however the precise cellular significance of this phenomenon is not fully understood. Continued efforts to characterize the regulation of these enzymes will provide important insights to their cellular function.

Recent structural characterisations of both type I and type II Acots has provided important functional information for both classes of enzymes. Active sites, substrate specificity, and interactions that mediate inter-domain interactions have been described. With the high degree of sequence similarity within each class of Acots, it is likely that homology modelling of the remaining members can provide functional hypotheses that can be tested experimentally.
References – these need to be sorted – check that references like Bloggs et al showed ...


