Regulation of acetyl-CoA carboxylase

R.W. Brownsey, A.N. Boone, J.E. Elliott, J.E. Kulpa and W.M. Lee
Department of Biochemistry and Molecular Biology and The Diabetes Research Group of the Life Sciences Institute, The University of British Columbia, 2350 Health Sciences Mall, Vancouver, BC, Canada, V6T 1Z3

Abstract
Acetyl-CoA carboxylase (ACC) catalyses the formation of malonyl-CoA, an essential substrate for fatty acid synthesis in lipogenic tissues and a key regulatory molecule in muscle, brain and other tissues. ACC contributes importantly to the overall control of energy metabolism and has provided an important model to explore mechanisms of enzyme control and hormone action. Mammalian ACCs are multifunctional dimeric proteins (530–560 kDa) with the potential to further polymerize and engage in multiprotein complexes. The enzymatic properties of ACC are complex, especially considering the two active sites, essential catalytic biotin, the three-substrate reaction and effects of allosteric ligands. The expression of the two major isoforms and splice variants of mammalian ACC is tissue-specific and responsive to hormones and nutritional status. Key regulatory elements and cognate transcription factors are still being defined. ACC specific activity is also rapidly modulated, being increased in response to insulin and decreased following exposure of cells to catabolic hormones or environmental stress. The acute control of ACC activity is the product of integrated changes in substrate supply, allosteric ligands, the phosphorylation of multiple serine residues and interactions with other proteins. This review traces the path and implications of studies initiated with Dick Denton in Bristol in the late 1970s, through to current proteomic and other approaches that have been consistently challenging and immensely rewarding.

Introduction
The storage of body fat provides a crucial reserve to sustain energy metabolism during starvation, hibernation and migration, as well as providing thermal insulation and other functions. However, excess body fat, especially in certain adipose depots and other tissue sites, can be maladaptive in humans, substantially increasing the risk of diabetes and cardiovascular diseases. The incidence of obesity has been rising sharply in recent decades in many populations, perhaps most ominously in adolescents, portending poor health outcomes later in life [1]. The work described in this review concerns ACC (acetyl-CoA carboxylase; EC 6.4.1.2), an enzyme that has a strong impact on lipid storage and overall energy metabolism. ACC is crucial because it catalyses the formation of malonyl-CoA, an essential substrate for fatty acid synthase and chain elongation systems and also a potent inhibitor of fatty acid oxidation; the overall significance of ACC has been underlined by recent studies with knockout mice [2,3]. Here, we outline the properties of ACC isoforms in order to understand how defects in ACC isoform expression or control mechanisms might contribute to the pathophysiology of obesity, insulin resistance and diabetes.

The ACC reaction
ACC was discovered by Salih Waki and his co-workers in the late 1950s and is one of a family of biotin-dependent enzymes with related structural and catalytic properties [4,5]. Biotin is covalently attached, by holo-carboxylase synthetase, to the ε-amino group of a critical lysine residue of apo-ACC and acts as a carboxyl 'carrier' in the two-step ACC reaction [6]. The first partial reaction (I) is ATP-dependent and occurs at the biotin carboxylase active site, forming carboxy-biotinyl-ACC. In the second partial reaction (II), the carboxy group is transferred from biotin to acetyl-CoA, generating malonyl-CoA:

\[
\text{ATP} + \text{bicarbonate} + \text{biotinyl-ACC} \rightarrow \text{ADP} + \text{P}_i + \text{carboxy-biotinyl-ACC} \quad \text{(Reaction I)}
\]
\[
\text{Carboxy-biotinyl-ACC} + \text{acetyl-CoA} \rightarrow \text{biotinyl-ACC} + \text{malonyl-CoA} \quad \text{(Reaction II)}
\]

The malonyl-CoA produced by ACC is an essential substrate for fatty acid synthase, notably in fat, liver and lactating mammary gland. Malonyl-CoA is also required by chain elongation systems of the endoplasmic reticulum in many tissues, with fatty acids \( \geq C_{20} \) having many important functions in the form of eicosanoids and glyco-, sphingo- and glycerolipids [7]. Malonyl-CoA also contributes to the regulation of fatty acid oxidation by inhibiting CPT-I (carnitine palmitoyl-CoA transferase-I), thereby co-ordinating the rates of hepatic fatty acid synthesis, β-oxidation and ketogenesis [8]. Generation of malonyl-CoA is also significant in the regulation of fatty acid oxidation in rodent heart and skeletal muscle, although the importance of malonyl-CoA in human skeletal muscle in vivo has been debated, because in some studies the rates of β-oxidation correlated poorly with total tissue concentrations of malonyl-CoA, while in other studies...
exercise did induce AMPK (AMP-activated protein kinase) activation and ACC phosphorylation in skeletal muscle [9–12]. On balance, these studies provide evidence that ACC activation may provide a powerful mechanism to contribute to the glucose–fatty acid cycle, controlling the relative contributions of lipids and carbohydrates to energy metabolism. Debate also continues concerning the possibility that palmitoyl-CoA may play a role in nutrient-induced insulin secretion in pancreatic β-cells [13]. For a variety of reasons, defining the key properties of ACC is therefore important in understanding the overall control of energy metabolism.

**ACC structure and isoforms**

The structure of ACC is remarkable in several respects and hints at the complexity of its regulatory properties. The catalytic centres and biotin prosthetic group of prokaryotic carboxylases are generally located on separate polypeptides encoded by distinct genes, whereas multifunctional ACC polypeptides have emerged in yeast, animals and higher plants, including two major animal isoforms designated ACC-1 and ACC-2 [6,14,15].

ACC-1 subunits have a predicted mass of 265 kDa and form tightly associated dimers with low catalytic activity that can further associate in vitro into highly active filamentous polymers, up to 4000 Å in length (1 Å = 0.1 nm) [6,16]. ACC dimers and polymers also exist in equilibrium physiologically, the proportion of active polymers being rapidly increased in fat and liver cells exposed to insulin and decreased under catabolic conditions [17].

ACC-2 was first discovered in rat heart [18] and the subunits (~280 kDa) are larger than those of ACC-1, mostly due to an N-terminal extension; however, as first shown by peptide mass fingerprint analysis, the two isoforms show many additional differences [19]. The N-terminal sequence of ACC-2 contains a potential mitochondrial targeting motif and, although ACC-2 is recovered in cytosolic fractions following cell homogenization, the expressed N-terminal domain appears to co-localize with mitochondria in intact cells [20], an observation that is supported by the distinct functions ascribed on the basis of gene knockouts [2,21]. In addition to the major ACC-1 and ACC-2 isoforms, variants of both isoforms have been detected that differ in the presence or absence of specific sequences [15]. Three-dimensional structures have been reported for the biotin carboxylase and biotin carboxyl carrier protein of *Escherichia coli* [22,23] and for a eukaryotic carboxyltransferase domain [24]. The structure of intact mammalian ACC has not yet been solved, largely because of problems in expressing and purifying sufficient protein. At a higher level of organization, it is possible that ACC may exist in a complex with other proteins and the evidence for this concept is described in more detail below.

**Control of ACC expression**

ACC-1 is highly expressed (10–50 µg/g of wet weight) in white and brown fat, liver and lactating mammary gland, with expression being inhibited during starvation or insulin deficiency and restored by refeeding a low-fat diet or by insulin treatment [25] (Table 1). The ACC-1 gene is under the control of at least three promoters, designated PI–PIII, with distinct roles in constitutive or inducible expression in response to a variety of factors including glucose, insulin, thyroid hormone, catalytic hormones and leptin [26–28]. Among the key transcription factors, SREBP1c (sterol-regulatory-element-binding protein 1c) plays a major role in controlling genes for ACC-1 and other lipogenic enzymes, in adipogenesis and in nutritional regulation [29]. Other transcription factors that contribute to the control of ACC expression include liver X receptor, retinoid X receptor, PPARs (peroxisome-proliferator-activated receptors), FOXO (forkhead box O) and PGC (PPAR co-activator) isoforms [15,30].

ACC-2 is expressed at low levels (1–2 µg/g of wet weight) in heart, skeletal muscle and various other cell types including human (but not rat) white fat [18]. The highest concentrations of ACC-2 are found in liver, where it represents approx. 20–25% of total hepatic ACC [19]. The expression of ACC-2 is increased during the differentiation of heart and skeletal-muscle cells, with a parallel decline in expression of ACC-1 [31]. In liver cells, in contrast, the expression of ACC-2 appears to parallel that of ACC-1, being decreased during starvation and restored on refeeding [32]. ACC-2 may have additional significance in humans because it is also expressed in human white adipose tissue. ACC-2 expression is also sensitive to leptin, the effects of which are mediated by PPARα, MyoD, Myf4 and Myf6 [33,34].

**Allosteric control of ACC**

Exposure of cells to insulin in vivo or in vitro leads to substantial activation of ACC-1 within 2–5 min [35,36] and the inhibition of ACC-1 following exposure of cells to catecholamines or glucagon is equally rapid [37,38]. The activity of ACC-1 is acutely controlled by allosteric modulators and by the phosphorylation of four or more serine residues. Among the allosteric effectors of ACC-1, citrate and other carboxylic acids activate the enzyme by promoting polymerization [6,16] (Table 1). Glutamate also activates ACC-1, suggesting that signals from amino acid metabolism also have a bearing on fatty acid metabolism [39]. Malonyl-CoA, free CoA and fatty acyl-CoA esters are all potentially important inhibitors of ACC-1 [40,41].

The effects of insulin on ACC-1 cannot be accounted for simply by changes in cellular concentrations of allosteric regulators; for example, insulin has little effect on total or cytosolic citrate concentrations in fat or liver cells [36]. In adipose tissue, insulin causes a decrease in total fatty acyl-CoA concentration; however, the effective cytosolic concentration of fatty acyl-CoA esters is difficult to determine because of buffering by cellular binding proteins [42]. In contrast, in oxidative tissues such as the heart, increases in malonyl-CoA may be associated with increases in fatty acyl-CoA concentrations, as a consequence of the inhibition of CPT-1 [43]. Finally, while allosteric ligands certainly influence the activity of ACC-1, the effects of insulin and catecholamines are still apparent following enzyme isolation, thus
Table 1 | Summary of control features of mammalian ACC

<table>
<thead>
<tr>
<th>Feature</th>
<th>Comments</th>
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<tbody>
<tr>
<td>Isoforms and subtypes</td>
<td>Two major isoforms, ACC-1 and ACC-2, each with several possible variants, depending on mRNA splicing</td>
</tr>
<tr>
<td>Expression</td>
<td>Absolute amount and isofom ratio is tissue-specific.</td>
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<tr>
<td></td>
<td>Developmental controls important in heart, mammary gland etc.</td>
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<td></td>
<td>Hormone and nutrient effects mediated by three promoters and multiple transcription factors</td>
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<td></td>
<td>Protein maturation requires biotin and holo-carboxylase synthetase</td>
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<td></td>
<td>Protein stability varies over the range 24–72 h</td>
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<tr>
<td>Allosteric control</td>
<td>ACC dimers exist in ‘active’ and ‘inactive’ conformations</td>
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<tr>
<td></td>
<td>‘Active’ dimers of ACC-1 can undergo extensive polymerization</td>
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<tr>
<td></td>
<td>ACC-2 dimers, alone, polymerize very little</td>
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<td></td>
<td>ACC-2 can participate in large polymers in association with ACC-1</td>
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<tr>
<td></td>
<td>Activating ligands include citrate, glutamate and other dicarboxylic acids</td>
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<td></td>
<td>Inhibitory ligands include short- and long-chain acyl-CoA esters</td>
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<tr>
<td>Covalent control</td>
<td>ACC-1 and ACC-2 contain up to eight phosphorylated residues</td>
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<tr>
<td></td>
<td>No sites on ACC-2 have yet been defined</td>
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<tr>
<td></td>
<td>ACC-1 is inhibited by phosphorylation (Ser^{79}, Ser^{1200}, and Ser^{1215}) by AMPK</td>
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<td></td>
<td>Insulin activation of ACC-1 is associated with dephosphorylation of AMPK sites and increased phosphorylation of other site(s)</td>
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<tr>
<td></td>
<td>Several phosphorylation sites have unknown functions, including those phosphorylated by CK2, PKC and CaMK (Ca^{2+}/calmodulin-dependent protein kinase)</td>
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<tr>
<td>Protein–protein interactions</td>
<td>Properties of homogeneous ACC do not account for enzyme activity in intact cells</td>
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<td>Genome-wide screens provide evidence for ‘ACC-associated proteins’ and possible ‘fatty acid metabolons’</td>
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<tr>
<td></td>
<td>Candidates include enzymes of fatty acid metabolism, molecular chaperones, cytoskeletal proteins and others</td>
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<td></td>
<td>Interactions may dictate cellular localization, activity and control properties</td>
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Providing evidence for the importance of additional control mechanisms [36]. ACC-2, like ACC-1, is activated by citrate and glutamate but ACC-2 does not polymerize as readily as ACC-1, perhaps reflecting properties related to localization at the outer mitochondrial membrane. Regardless of polymerization, it has been argued that allosteric control of ACC-2 by citrate is crucial in skeletal muscle [44].

Although the responses of ACC to citrate and other carboxylic acids have been known for many years, the allosteric sites have not been defined. In studies of other citrate-binding proteins, notably phosphofructo-1-kinase and carboxylate transporters, PLP (pyridoxal phosphate) was found to act as a structural analogue of citrate and has been used to probe the citrate-binding sites [45]. We have recently demonstrated that PLP inhibits both ACC isoforms, appears to compete with citrate for binding to the allosteric site and can be covalently coupled with the enzyme subunits [46]. Although PLP can potentially interact with the ε-amino group of many lysine side chains, such non-selective interactions require substantially higher concentrations than those required to inhibit ACC activity. Furthermore, the binding of PLP to ACC is observed in the presence of a large excess of serum albumin and occurs to a stoichiometry of approx. 0.5–1.0 mol of PLP/mol of ACC, despite the fact that the ACC sequence contains more than 100 lysine residues. Identification of the critical lysine will be a key goal in defining the citrate-binding site, although additional determinants of citrate binding could be widely separated in the ACC sequence, in which case the full definition of the allosteric site will require knowledge of the three-dimensional structure. The ability of PLP to inhibit ACC might also provide the basis for the refinement of a distinct class of ACC inhibitors, those available so far being directed at the active site [47].

Differential control of ACC-1 and ACC-2 by multiple-site phosphorylation

Experiments involving metabolic labelling with [^{32}P]phosphate led to the demonstration that ACC-1 is phosphorylated on at least four hormone-responsive sites in fat and liver cells. Treatment of these cells with adrenaline or glucagon leads to rapid phosphorylation and inactivation of ACC-1. The effects of the inhibitory hormones are mediated by cAMP, but the phosphorylation of ACC-1 at Ser^{79}, Ser^{1200} and Ser^{1215} is carried out largely by AMPK rather than by PKA (protein kinase A) [38] (see Table 1).

The activating effects of insulin on ACC-1 are complex and still unresolved. Insulin reverses the inhibition caused by AMPK in hepatoma and heart cells [48], although this effect has not been observed in primary fat or liver cells and appears to be independent of changes in concentration of cAMP or 5′-AMP. In fact, full activation of ACC-1 is also accompanied by ‘increased’ phosphorylation by an insulin-activated protein kinase [49]. Like other metabolic effects, the activation of ACC by insulin is blocked by inhibition
of the PI3K (phosphoinositide 3-kinase) signalling pathway, but the relevant ‘ACC kinase’ has not been identified [50]. In our own studies over the past 2 years, we have found that PKB and atypical PKC isoforms, among the major kinases activated by PI3K, phosphorylate ACC poorly or act on sites not seen in response to insulin.

Purified ACC-2, like ACC-1, is a substrate for AMPK, and is inactivated during ischaemic perfusion of hearts and in electrically stimulated or exercising skeletal muscle, in parallel with activation of AMPK [51,52]. However, in well-oxygenated muscle tissues stressed by increasing workloads or adrenergic stimulation, ACC is either unaffected or inactivated despite the fact that 5′-AMP concentrations are not consistently increased [53]. Although AMPK might be activated by AMP-independent mechanisms, it is also possible that other kinases are involved in ACC-2 inhibition. Consistent with this idea, ACC-2 is a far better substrate for PKA than ACC-1 and is rapidly phosphorylated by PKA within intact cardiac myocytes treated with isoproterenol or in endothelial cells treated with leptin [54,55]. Likewise, α-adrenergic stimulation suppresses ACC activity in rat liver and skeletal muscle, suggesting that additional ACC kinases may be physiologically important [56,57].

**ACC polymers, complexes or fatty acid ‘metabolons’?**

It has long been speculated that metabolic enzymes may be organized in complexes or ‘metabolons’, especially considering that high intracellular protein concentrations favour protein–protein interactions [58]. Significantly, the properties of highly purified ACC do not adequately account for enzyme function within intact cells, suggesting that additional intracellular factors may facilitate ACC function. For example, maximal rates of fatty acid synthesis are in the range 10–20 μmol of acetyl units/h per g of wet weight in rat liver and adipose tissue, *in vitro* [35]. These rates require essentially full activation of available ACC, despite the fact that tissue concentrations of citrate and long-chain acyl-CoA esters are far from optimal. Some years ago, we obtained evidence for a regulatory protein that co-purified with ACC [59], and since then, protein interaction studies have revealed a number of proteins that associate with ACC in screens of ‘model’ organisms, notably *Saccharomyces cerevisiae*, *E. coli*, *Drosophila melanogaster* and *Caenorhabditis elegans* [60–62]. For example, these studies provide evidence that yeast ACC interacts strongly with Sit4, Cct5 and a protein designated YLR386W. Sit4 is the catalytic subunit of the yeast protein serine/threonine phosphatase that is related to PP2A (protein phosphatase 2A) and human PP6 (protein phosphatase 6); it plays a significant role in cell cycle progression and is inhibited by ceramide. Similarly, the interaction of ACC with the yeast chaperonin Cct5 and the *E. coli* heat-shock protein related to HSP70 (heat-shock protein 70) raises the possibility that a mammalian chaperone might influence ACC. Considering the nature of ACC polymerization and depolymerization, it is not unreasonable to predict a mediating role for a chaperone. Interestingly, one screen using 14-3-3 protein sequences as ‘baits’ has provided evidence for interactions between ACC and the lipogenic enzyme ATP citrate-lyase. In light of these studies, we are currently using proteomic techniques to identify ACC-associated proteins in mammalian cells, the overall goal being to reproduce the *in vivo* properties of ACC.

Of course, the possibility of non-specific protein binding to ACC is a significant potential problem and it will be important to establish that any candidate proteins play a significant role within intact cells. Furthermore, there are few clues currently available to guide the search for proteins that interact with ACC-2, because the classic ‘model’ organisms appear not to express an equivalent form of the enzyme.

I (R.W.B.) acknowledge the contributions of a number of graduate students, postdoctoral fellows and other colleagues, in addition to the co-authors, who have enriched the work described above. To a large extent, they appear as co-authors in the cited references. In particular, given the occasion of this presentation, it is important to recognize that my interests in this fascinating enzyme would never have come about had it not been for the happy accident of working in Bristol and, in particular, in ‘M101’ with Dick Denton, soon after he and Andrew Halestrap had set the scene with their seminal observations describing the hormone sensitivity of ACC.

**References**
