It is clear that down-regulation of a specific component of lipid metabolism not only alters seed storage lipid content, but also affects other components of that pathway. The effects go even further in potentially altering the allocation of resources within the seed and lowering the proportion of seeds reaching maturity. It is clear that there must be some cross-talk between signalling pathways within the cell; these pleiotropic effects obviously have great biological significance, as they give a clear indication that cellular events are connected. Future proteome and transcriptome analyses should help to elucidate the potential connections between these different components of metabolism and link metabolism to cell biology. These plants will form a useful resource for future metabolic flux analysis studies that are in progress.

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References


Abstract

Acetyl-CoA carboxylase (ACC) plays a critical role in the regulation of fatty acid metabolism and its two isoforms, ACCα and ACCβ, appear to have distinct functions in the control of fatty acid synthesis and fatty acid oxidation, respectively. They are regulated by similar short-term mechanisms of allosteric activation by citrate, and reversible phosphorylation and inactivation, and there is clearly interaction between these mechanisms. AMP-activated protein kinase is the important physiological ACC kinase for both isoforms and yet there is a potential physiological role for cAMP-dependent protein kinase in the hormonally mediated inactivation of ACCα, and phosphorylation of ACCβ in its unique N-terminus.

Key words: AMP-activated protein kinase, cAMP-dependent protein kinase, fatty acid oxidation, fatty acid synthesis.

Abbreviations used: ACC, acetyl-CoA carboxylase; AlCAR, 5-aminoimidazole-4-carboxamide ribonucleoside; AMPK, AMP-activated protein kinase; CPT1, carnitine palmitoyltransferase-1; PKA, cAMP-dependent protein kinase.

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Regulation of mammalian acetyl-CoA carboxylase

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Acetyl-CoA carboxylase (ACC) isoform structure and function

ACC catalyses the carboxylation of acetyl-CoA to malonyl-CoA, a major precursor for fatty acid synthesis [1], but it is also a crucial regulator of mitochondrial fatty acid β-oxidation through its inhibition of carnitine palmitoyltransferase-1 (CPT1) [2]. The regulation of ACC, through a complex interaction of short- and long-term mechanisms, plays a key role in the control of both the synthesis and the oxidation of fatty acids.

In mammals, ACC is a multifunctional enzyme with biotin carboxyl carrier protein, biotin carboxylase and carboxyl transferase domains all contained within a single polypeptide chain [1]. A large body of evidence, including purification [3–6], immunological analysis [7] and cDNA cloning [8–11], has revealed two major isoforms of ACC with molecular masses of approx. 265 kDa (ACC1 or ACCα) and 280 kDa (ACC2 or ACCβ). The two isoforms are distinct gene products with the ACCα gene localized to human chromosome 17 [9] and the ACCβ gene localized to human...
chromosome 12 [10]. The major difference between the isoforms is the extended N-terminus of ACCβ. Outside this region, the isoforms exhibit considerable identity and have the same domain structure responsible for catalytic activity [10]. Despite the identity, slight differences in the kinetic parameters of the two isoforms have been reported, with ACCβ exhibiting lower $K_m$ values for ATP and HCO$_3$ and a higher $K_m$ value for acetyl-CoA [6,7]. Both isoforms are activated by citrate with different $K_v$ values [6,7].

The distinct tissue distribution of the isoforms may be a better indicator of possible functional differences. In lipogenic tissues, such as white adipose tissue and lactating mammary gland, ACCα is the major form of ACC expressed. In oxidative tissues, such as heart and skeletal muscle, ACCβ predominates. In liver, where both fatty acid synthesis and oxidation are important, there is significant expression of both isoforms [6,7,10]. Thus ACCβ may be involved in the control of fatty acid oxidation, while ACCα controls fatty acid synthesis. There has been much speculation as to whether or not the unique N-terminal peptide of ACCβ could function to anchor ACCβ to the mitochondrial membrane, where it could produce malonyl-CoA in close proximity to the active site of CPT-1. There have been previous reports of mitochondria-associated forms of ACC [12], but these have not always been supported [13,14]. Overexpression of the N-terminal peptide of ACCβ [15] and expression of an antisense gene to the ACCβ N-terminus [16] in H9c2 cardiomyocytes have suggested a specific role in fatty acid oxidation, but not fatty acid synthesis. The clearest evidence so far has come from Wakil and colleagues, who used immunofluorescent microscopic analysis with anti-ACCβ antibodies, and green fluorescent protein fused to the C-terminus of the ACCβ N-terminal domain, to show a mitochondrial location of ACCβ [17]. Furthermore, they have reported significantly lowered levels of malonyl-CoA and elevated rates of fatty acid oxidation in heart and muscle of ACCβ knockout mice [18].

The minimum molecular mass of native ACC suggests that the smallest form that occurs in vivo is a dimer. Immunoprecipitation of ACC with specific ACCα or ACCβ antibodies suggests that this could be a homodimer or a heterodimer of the two different isoforms [7,19]. This dimer is frequently referred to as the protomeric form (12–13 S) which, in the presence of high concentrations of citrate, forms polymers (>40 S) of up to 30 protomers in a linear array [3]. There is some evidence that the polymerization of the enzyme may be an integral part of its regulation.

### Short-term regulation of ACC

Significant changes in the concentration of ACC do not occur rapidly, since the half-life of this enzyme varies from 1 to 3 days under different physiological conditions [20]. However, there is nutritional and hormonal control of ACC gene expression, as reviewed elsewhere [21]. The short-term control of ACC activity is achieved via allosteric regulation and reversible phosphorylation and inactivation. These mechanisms appear to work for both isoforms, but with some subtle differences.

### Allosteric regulation by citrate and fatty acyl-CoA

Citrate is a feedforward activator of ACC with a $K_v$ of approx. 2 mM [22], so that the physiological range of cellular citrate concentrations (0.1–1 mM) is a potential allosteric activator in vivo. However, cell citrate concentrations do not always change in parallel with the activity of ACC [23,24]. This is probably due to the potential overriding influence of phosphorylation. Citrate causes polymerization of ACC (see above), although it appears that the activation by citrate precedes polymerization [25]. However, there is evidence for protomer into polymer transition in vivo, where the effects of insulin treatment on adipocyte ACC, and of insulin injection or refeeding starved rats on hepatic ACC, include the transition of ACC to a high-molecular-mass polymeric form, as judged by size exclusion chromatography [26,27]. Insulin and glucose inhibit fatty acid oxidation in rat and human skeletal muscle via an increase in malonyl-CoA concentration, without a stable, measurable change in ACC activity [28,29]. There is, however, a strong positive correlation between malonyl-CoA and increased concentrations of cell citrate and malate (that would promote citrate efflux from the mitochondria). This has led Ruderman and colleagues to propose that allosteric activation of ACCβ by citrate rather than a change in its phosphorylation state is responsible for the increased malonyl-CoA levels [28,29]. In acini isolated from lactating rat mammary gland we have observed a 115±21 % ($n = 4$) increase in ACC activity, accompanying a 3-fold increase in cell citrate concentration, following treatment with acetocetate. $^{32}$P-labelling of cells revealed...
that ACC from treated cells was less phosphorylated than that from control cells. Phosphorylation and allosteric activation are clearly interactive mechanisms. Phosphorylation of pure ACC by cAMP-dependent protein kinase (PKA) or AMP-activated protein kinase (AMPK) both result in decreased sensitivity of ACC to citrate. Conversely, a highly polymerized and citrate-dependent form of rat liver ACC has been identified as being relatively dephosphorylated [27]. We have seen that citrate has a small inhibitory effect (25 ± 1.9 %, n = 4) on the rate of phosphorylation by AMPK of the SAMS peptide (a synthetic 15 amino acid peptide containing the sequence around Ser79 on ACC). However, citrate caused 77 ± 3.4 % (n = 4) inhibition of the rate of phosphorylation of native ACC by AMPK. We conclude that the polymerization of ACC in the presence of citrate protects it from phosphorylation and that this could be the basis of the effect of raised citrate concentrations on ACC in acini in response to acetoacetate.

Long-chain fatty acyl-CoA esters are potent feedback inhibitors of ACC, at concentrations below the critical micelle concentration [30]. The $K_i$ values for saturated and unsaturated fatty acyl-CoA with chain lengths of 16 or more carbon atoms vary from 1 to 150 nM [30]. There is good evidence that exogenous fatty acids inhibit lipogenesis, but little evidence that this is through the allosteric inhibition of ACC [23,31]. In rat skeletal muscle, refeeding after a fast increases malonyl-CoA and decreases fatty acid oxidation, which has been attributed to a decrease in fatty acids that releases allosteric inhibition of ACC [32].

### Phosphorylation and inactivation of ACC in vitro

The phosphorylation of ACC is crucial to its proposed role in the control of fatty acid oxidation. ACC$\beta$ from rat liver or heart is a better substrate for PKA than ACC$\alpha$ [5,19], which is surprising, given that ACC$\beta$ lacks an equivalent to the major PKA phosphorylation site at Ser$^{160}$ in rat ACC$\alpha$ [11]. This may be explained by the existence of four or five consensus sequences for PKA phosphorylation sites in the N-terminal extension of ACC$\beta$ [11]. There are conflicting reports that PKA phosphorylation of ACC$\beta$ in vitro produces significant inactivation [19] or has no effect on activity [35,36]. It is possible that phosphorylation of the N-terminal sites controls the association of ACC$\beta$ with the mitochondrial membrane and thus regulates CPT1 and fatty acid oxidation through a targeting mechanism. ACC$\beta$ and ACC$\alpha$ in rat liver appear to be equally good substrates for AMPK in vitro and phosphorylation of ACC$\beta$ by AMPK causes inactivation [19,36] in the form of an increased $K_i$ for citrate and decreased $V_{\text{max}}$ [36]. Ser$^{318}$ in ACC$\beta$ appears to be the equivalent of Ser$^{79}$, the major phosphorylation site of AMPK in ACC$\alpha$ [11]. It is noteworthy that tryptic peptide maps of ACC$\beta$, phosphorylated by PKA and AMPK, show significant differences [35].

### Physiological phosphorylation and inactivation of ACC in vivo

The phosphorylation state of ACC in rat liver changes with nutritional and hormonal status. There is a diurnal rhythm [37]; ACC is also phosphorylated and inactivated in response to prolonged food withdrawal [27,38], feeding rats
that AMPK is the physiological ACC kinase, it is high-fat diet [37], glucagon treatment of isolated hepatocytes [39] or glucagon administration to whole rats [40]. These physiological phosphorylations and inactivations of ACC are characterized by a large significant increase in the $K_v$ for citrate and an equally large decrease in the $V_{\text{max}}$ of the enzyme. Such changes in ACC $V_{\text{max}}$ values can only be explained by the effects of phosphorylation by AMPK, and not PKA. In support of the fact that AMPK is the physiological ACC kinase, it is Ser$^79$ that becomes phosphorylated in rat liver ACC in response to in vivo manipulations [37] and in hepatocytes in response to glucagon [39].

Purification, cloning and expression of rat liver AMPK has revealed that it is heterotrimeric and consists of catalytic α and regulatory β and γ subunits [41,42]. Multiple isoforms of each subunit have been identified (α1 and α2; β1 and β2; γ1, γ2 and γ3) and individual roles for these are under investigation. Witters and colleagues have reported that the α1 isoform specifically phosphorylates rat liver ACCα and ACCβ [43], yet Lopaschuk and colleagues report that the α2 isoform co-precipitates with immune complexes of ACCβ from rat heart [19]. The major role of AMPK in the cell is to monitor its energy status and regulate the ATP-consuming and ATP-producing pathways accordingly. Thus when ATP is depleted and AMP concentrations rise, AMPK is activated by a multi-faceted mechanism comprising: (i) direct allosteric activation of AMPK by AMP; (ii) phosphorylation and activation by an upstream AMPK-kinase in a reaction that is stimulated by AMP; (iii) AMP inhibition of the dephosphorylation and inactivation of AMPK [41,42,44]. As ACCα catalyses the energy-utilizing pathway of fatty acid synthesis in liver, it is a prime target for AMPK in times of oxidative stress. Thus, phosphorylation and inactivation of ACC have been demonstrated in liver, in response to anoxia [37], in hepatocytes, in response to raising AMP through incubation with ATP depletors [44], and in response to 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), a cell-soluble precursor to ZMP (AICAR monophosphate) that is an analogue of AMP [45].

The mechanism of hormonally or nutritionally mediated phosphorylation of ACC by AMPK is unclear. Glucagon treatment of hepatocytes [39] or adrenaline treatment of adipocytes [46] results in the phosphorylation of Ser$^79$ on ACCα by AMPK. Pharmacological blockade of cAMP action in adipocytes prevented this AMPK-mediated phosphorylation of ACCα [46], yet PKA is not the upstream AMPK kinase that activates AMPK. One can only speculate that the effects of cAMP are mediated through changes in the activity of ACC phosphatase, or some mechanism that targets AMPK to ACC (or vice versa). Where changes in AMPK activity in response to hormones have been reported, these have been linked to inverse changes in insulin concentration [38] and a direct inhibition of AMPK activity in Fao Reuber hepatoma cells, in response to insulin, has been observed [47]. Most recently, leptin has been shown to activate AMPK in skeletal muscle, resulting in phosphorylation and inactivation of ACCβ and stimulation of fatty acid oxidation [48].

There is increasing evidence that AMPK can be activated in the cell in the absence of changes in the ATP:AMP ratio and it seems likely that a number of cell signalling pathways may converge upon the AMPK signalling cascade.

In times of low energy status, the role of AMPK is not only to inhibit energy-utilizing pathways, but also to stimulate the use of alternative fuels to provide energy. Thus, phosphorylation of ACCβ, a resultant decrease in malonyl-CoA and disinhibition of CPT1, and stimulation of fatty acid oxidation, is another important function of AMPK that is most prominent, and well-documented, in heart and skeletal muscle [49]. Heart and skeletal muscle contain an isoform of CPT1 that is more sensitive to inhibition by malonyl-CoA than the liver enzyme [2]. Therefore, rates of fatty acid oxidation have been shown to closely and inversely correlate with malonyl-CoA levels in rat cardiomyocytes [50], perfused working rat heart [51] and rat hindlimb [52] under a variety of conditions. The 3–4-fold higher rates of fatty acid oxidation in reperfused rat hearts following ischaemia are due to a dramatic decrease in malonyl-CoA concentration, caused by the phosphorylation and inactivation of ACCβ in response to activation of AMPK [53]. In rat skeletal muscle, treadmill exercise [52] or electrical stimulation [54] caused activation of AMPK and subsequent phosphorylation and inactivation of ACCβ leading to decreased malonyl-CoA levels. The changes in kinetic parameters of ACCβ in these situations were very comparable with those seen in response to ACCβ phosphorylation by AMPK in vitro [36,52,54]. These effects were mimicked by the incubation of isolated rat soleus muscles with the AMPK activator, AICAR. AICAR decreased ACCβ activity by 50% and malonyl-CoA levels by 40%, and increased the rate of fatty acid oxidation by 90% [55].
muscle, the more readily available energy store is phosphocreatine, and AMPK is inhibited by phosphocreatine and is, therefore, sensitive to the phosphocreatine/creatine ratio [56]. As rat skeletal muscle contracts and uses energy, temporal decreases in the phosphocreatine level and increases in AMP correlate with time courses of activation of AMPK and phosphorylation and inactivation of ACCβ [57]. Similar time-dependent changes correlating with increased fat oxidation have been observed in human skeletal muscle [58].

Conclusions
The two isoforms ACCα and ACCβ appear to have distinct roles in the regulation of fatty acid synthesis and fatty acid oxidation, respectively. They are regulated by similar short-term mechanisms of allosteric activation by citrate and inhibition by fatty acyl-CoA, and reversible phosphorylation and inactivation. AMPK is the important physiological ACC kinase for both isoforms and yet a role for PKA cannot be ruled out, in terms of both the cAMP-mediated inactivation of ACCα and the fact that ACCβ is such a good PKA substrate at sites presumed to be in the unique N-terminus.

References
Regulation of fatty acid synthesis and oxidation by the AMP-activated protein kinase

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Abstract
The AMP-activated protein kinase (AMPK) is a sensor of cellular energy charge and a "metabolic master switch". When activated by ATP depletion, it switches off ATP-consuming processes, while switching on catabolic pathways that generate ATP. AMPK exists as heterotrimeric complexes comprising catalytic \( \alpha \) subunits and regulatory \( \beta \) and \( \gamma \) subunits, each of which occurs as multiple isoforms. Rising AMP and falling ATP, brought about by various types of cellular stress (including exercise in skeletal muscle), stimulate the system in an ultrasensitive manner. Acetyl-CoA carboxylase (ACC) exists in mammals as two isoforms, termed ACC-1 and ACC-2 (also known as ACC-\( \alpha \) and ACC-\( \beta \)). AMPK phosphorylates and inactivates both isoforms at the equivalent site. Knockout mice, and other approaches, suggest that the malonyl-CoA produced by ACC-2 is exclusively involved in regulation of fatty acid oxidation, whereas that produced by ACC-1 is utilized in fatty acid synthesis. Activation of AMPK by cellular stress or exercise therefore switches on fatty acid oxidation (via phosphorylation of ACC-2) while switching off fatty acid synthesis (via phosphorylation of ACC-1). The *Drosophila melanogaster* genome contains single genes encoding homologues of the \( \alpha \), \( \beta \) and \( \gamma \) subunits of AMPK (DmAMPK) and of ACC (DmACC). Studies in a *Drosophila* embryonic cell line show that DmAMPK is activated by stresses that cause ATP depletion (oligomycin, hypoxia or glucose deprivation) and that this is associated with phosphorylation of the site on DmACC equivalent to the AMPK sites on mammalian ACC-1 and ACC-2. This is abolished when expression of DmAMPK is ablated using an RNA interference approach, proving that DmAMPK is necessary for phosphorylation of DmACC in response to ATP depletion.

Key words: acetyl-CoA carboxylase, *Drosophila melanogaster*, AMPK, ACC-1, ACC-2, ACC-\( \alpha \), ACC-\( \beta \), ACC-\( \gamma \), ACC-P, ACC-Py. The genome contains single genes encoding homologues of the catalytic subunits of AMPK.