Acetyl-CoA carboxylase-α: Gene structure-function relationships

M. T. Travers and M. C. Barber

Hannah Research Institute, Ayr, KA6 5HL, United Kingdom

ABSTRACT: Acetyl-CoA carboxylase-α (ACC-α) is a key enzyme in the regulation of fatty acid synthesis and is subject to both acute control, via reversible phosphorylation, and chronic control that results in the regulation of synthesis of the enzyme. The gene for ACC-α is expressed ubiquitously, but expression is highest in lipogenic tissues: adipose, liver, and lactating mammary gland. These tissues demonstrate a metabolic adaptation to changing physiological demands; for example, during lactation fatty acid synthesis in adipose tissue is markedly repressed, resulting in the partitioning of lipogenic precursors to the mammary gland. Lipogenic tissues can also exhibit dysfunctions that result in excess fat deposition in farm animals and obesity in humans. Transcription of the ACC-α gene is initiated from multiple promoters in a tissue-specific fashion. Promoter II (PII) transcripts are present in all tissues, whereas promoter I (PI) transcripts are principally restricted to adipose tissue. We have also identified an additional promoter (PIII) that is also expressed in a tissue-restricted manner. All three promoters are modulated by the physiological state of an animal, suggesting that each promoter possesses enhancer domains that are targets for cell-specific signaling pathways and act in concert with the basal transcriptional machinery to regulate expression of the gene. For example, expression of the ACC-α gene is increased in mammary gland during lactation concomitant with the increase in the rate of fatty acid synthesis; in sheep this arises through induction of both PII and PIII promoter activities. Conversely, the ACC-α gene is repressed in sheep adipose tissue during lactation, and this occurs primarily through inactivation of PI, although PII activity is also repressed. This may arise in part from a change in sensitivity of the tissue to insulin. Analysis of the structure and function of the various promoters of ACC-α is presented with a view to determining the molecular basis of the modulation of expression of this gene in lipogenic tissues.

Key Words: Adipose Tissue, Insulin, Mammary Glands

©2001 American Society of Animal Science. All rights reserved.

J. Anim. Sci. 79(E. Suppl.):E136–E143

Introduction

In higher animals, increases in the cellular activity of acetyl-CoA carboxylase (ACC), the flux-determining enzyme in the synthesis of fatty acids, results in an increased production of malonyl-CoA. This plays a pivotal role in orchestrating the partitioning of nutrients between liver and peripheral tissues. This is part of an adaptive strategy to respond to the physiological demands created by states such as feeding, starvation, and lactation (Zammit, 1996; Loftus et al., 2000).

During lactation ACC plays a key role in facilitating the delivery of fatty acid precursors to the mammary gland for use in the synthesis of milk fat. This is achieved through repression of ACC activity in adipose tissue and induction in mammary gland, in part through corresponding changes in the level of ACC mRNA in the two tissues (Barber et al., 1997) (Figure 1).

To meet these physiological demands, ACC activity is controlled acutely in a complex fashion involving allosteric modulation and multiple site-specific phosphorylation (Kim et al., 1989) and chronically through changes in gene expression (Kim and Tae, 1994). More recently ACC activity was found to be due to the expression of isozymes from two related genes, ACC-α and ACC-β. The ACC-α gene is expressed in all tissues but induced in lipogenic tissues (adipose tissue, liver, and lactating mammary gland) whereas ACC-β is chiefly confined to tissues adapted to fatty acyl β-oxidation (Abu-Elheiga et al., 1997). For ACC-α further diversity due to tissue-specific promoter usage and alternative exon splicing has added to this complexity. How this diversity in ACC at the gene, transcript, and protein levels is related to the function of different cell types and tissues with regard to their malonyl CoA and lipid requirements is a key question. This article will confine
Figure 1. Expression of acetyl-CoA carboxylase-α (ACC-α) mRNA in adipose tissue and mammary gland of sheep during pregnancy and lactation. The RNA in tissue homogenates was used in an RNase protection assay (Travers and Barber, 1999) using a riboprobe corresponding to a region of coding sequence common to all ACC-α transcripts. After quantifying the amount of protected mRNA, this was expressed per milligram of tissue DNA. The graphs represent the mean and standard error of five animals per point.

itself to aspects of the ACC-α gene about which information on the molecular biology and physiology is known in more detail.

ACC-α Gene Structure

The cDNA for ACC-α was first cloned from lactating rat mammary gland (Lopez-Casillas et al., 1988) and contained an open reading frame of 7,035 nucleotides encoding a protein of molecular weight 265 kDa; ACC-β is 280 kDa. Northern blot analysis showed the ACC-α cDNA to hybridize to an mRNA of 9.5 to 10 kb, and titration analysis indicated only one gene per haploid chromosome set, subsequently found to be located on chromosome 17q12 in humans (Abu-Elheiga et al., 1995). Using this cDNA and simple dot blot analysis of RNA the amount of ACC-α mRNA was found to be regulated by diet, diabetes, and lactation in the three major lipogenic tissues of the rat (liver, adipose, and mammary gland), and nuclear run-on analysis showed this regulation was at the level of transcription (Pape et al., 1988; Pape and Kim, 1989; Katsurada et al., 1990).

Further analysis of the 5′ ends of ACC-α transcripts in the lipogenic tissues using a primer extension technique demonstrated heterogeneity in the untranslated regions (UTR); different but overlapping sets of transcripts were found in lactating mammary gland and the liver and adipose tissue of starved/carbohydrate-refed rats, a treatment that results in induction of fatty acid synthesis (Lopez-Casillas et al., 1989; Lopez-Casillas and Kim, 1989). These 5′ UTR fell into two classes depending on the leader sequence used; Class 1 transcripts were found in liver and adipose tissue under lipogenic conditions and differ in the presence or absence of a 47-nucleotide sequence, and Class 2 transcripts that are found in all tissues, are increased in abundance in lactating mammary gland, and differ in the presence or absence of a 61-nucleotide and/or a 47-nucleotide sequence (Figure 2). A similar pattern of 5′ UTR heterogeneity and tissue distribution was also found for ovine ACC-α mRNA (Barber and Travers, 1995; Travers and Barber, 1999). Subsequent cloning of the rat ACC-α gene demonstrated that Class 1 and 2 transcripts are transcribed from distinct promoters, PI and PII, respectively (Luo et al., 1989), with differential splicing of the primary transcript to explain additional 5′ UTR heterogeneity; the 61- and 47-nucleotide sequences are encoded by exons 3 and 4, respectively. A similar genomic structure is also exhibited in the promoter region of the ovine ACC-α gene (Figure 2), with the exception that although there is sequence with good homology to exon 3, this sequence is not flanked by splice sites and does not seem to function as an exon. In addition, a third promoter, PIII, has been characterized in the ovine ACC-α gene (see below). To date the complete ACC-α gene structure from vertebrates is not known, although initial estimates from the distribution of exons in the promoter region of the rat (Luo et al., 1989) and sheep genes (Barber and Travers, unpublished data), and from overlapping human genomic
clones (Abu-Elheiga et al., 1995) would be in the region of 400 to 500 kb. With the release of the sequence from the Human Genome Project the full gene structure for Human ACC-α will soon be known.

Insight into the physiological relevance of the diversity of ACC-α transcripts initially came from observations of the tissue distribution and response to various physiological adaptations of these transcript types. In rats, starvation leads to a decrease in ACC enzyme activity in liver and adipose tissue, and refeeding a fat-free, high-carbohydrate diet results in induction of fatty acid synthesis in both tissues. In the adipose tissue of chow-fed animals the major mRNA contains exons 1, 4, and 5 (E1/4/5) with a smaller amount in which exon 4 has been spliced out (E1/5); PII transcripts are only expressed at a low level. After starvation for 48 h, PI transcript levels fall and then increase upon refeeding (Lopez-Casillas et al., 1991), demonstrating that PI is regulated by nutritional state and its expression is related to the fatty acid synthesis and storage capacity of the adipocyte. The PII transcripts have a ubiquitous tissue distribution in rats (Kim et al., 1996) and sheep (Travers and Barber, unpublished data) suggesting that their expression may be related to the endogenous fatty acid requirements of individual cell types. Interestingly, PII is also nutritionally regulated in the liver of starved-refed rats, in addition to PI (Lopez-Casillas et al., 1992).

The metabolic adaptations occurring in the lipogenic tissues during pregnancy and lactation that result in opposite effects on the transcription of the ACC-α gene in adipose tissue and mammary gland have led to investigations of whether differential use of the multiple promoters of this gene, and subsequent exon splicing to produce multiple mRNA, can be correlated with these changes and thus provide insight into the functional role of promoter and transcript diversity. Recent work in this area will be discussed further in the next section.

Hormonal Regulation of ACC-α Transcripts in Adipose Tissue and Mammary Gland

The amount of total ACC activity, the proportion of the enzyme in the active state, and the total levels of

![Figure 2](image)
ACCT-α transcripts are markedly suppressed in adipose tissue from 18-d lactating sheep compared to control (nonpregnant/nonlactating) animals (Vernon et al. 1987, 1991; Travers et al., 1997). This reduction in lipogenic potential occurs at a time when the serum insulin level is falling (0.69 ± 0.08 ng/mL in 18-d lactating animals compared to 1.22 ± 0.18 ng/mL in nonlactating animals) and that of growth hormone, which is potently antilipogenic, at least in adipose tissue in vitro, is raised (17.2 ± 3.9 ng/mL in 18-d lactating animals compared with 3.8 ± 0.7 ng/mL in nonlactating animals) suggesting these hormones may be implicated in this process (Vernon et al., 1981). In addition, the adipose tissue from lactating sheep when put into explant culture is initially refractory to stimulation with insulin and the synthetic glucocorticoid dexamethasone; no increase in ACC activity or ACC transcripts occurred until after 24 h of treatment (Travers et al., 1997).

As described above, four ACC-α mRNA (E1/4/5, E1/5, E2/4/5, and E2/5) generated from two promoters (PI and PII) are found in ovine adipose tissue; using an RNase protection assay to differentiate between and quantify these mRNA it has been shown that the repression of the ACC-α gene in lactation is largely due to a decline in PI transcripts compared with PII transcripts; levels of PI transcripts decreased to only 12% of the control value, whereas those from PII fell by less than half, with the proportion of the mRNA with or without exon 4 from each promoter remaining relatively unchanged (Travers and Barber, 1999) (Table 1). In contrast, when the effects of insulin and dexamethasone, both singly and together, on the levels of the different transcripts were assessed on adipose tissue from lactating animals, dexamethasone alone had no significant effect compared with no hormones, whereas insulin increased transcripts from both promoters 1.5- to two-fold. The combination of insulin and dexamethasone, although it had only a relatively slight effect on total PII transcripts (2.5-fold), increased both PI mRNA, 7- and 9.5-fold, respectively (Table 1). Thus, there is a synergy between insulin and glucocorticoid on ACC-α transcript levels, and the potentiation of insulin action by glucocorticoid is considerably more marked for PI than for PII transcripts (Travers and Barber, 1999). During lactation the serum glucocorticoid concentration rises, in contrast to the insulin concentration (Cowie et al., 1980), implying that the insulin effect per se may be responsible for the suppression of fatty acid synthesis, but that the glucocorticoid interaction may increase the sensitivity of PI, relative to PII, to the fall in insulin.

The RNase protection assay, however, only measures steady-state transcript levels that are the result of transcription rate and mRNA stability, and the effects of insulin and glucocorticoid could be on either or both of these variables. However, transfection of a fragment of the PI region within a luciferase reporter vector into primary ovine adipocytes in culture showed an increase in luciferase activity in these cells when treated with

### Table 1. (A) Expression of PI and PII ACC-α transcripts in adipose tissue during pregnancy and lactation and (B) in explants from lactating sheep at zero time and cultured with no hormones (NA), insulin (I), dexamethasone (D), or insulin plus dexamethasone (ID).p

<table>
<thead>
<tr>
<th>Tissue in vivo</th>
<th>Transcripts type</th>
<th>C</th>
<th>P</th>
<th>L</th>
<th>Transcripts type</th>
<th>O</th>
<th>NA</th>
<th>I</th>
<th>D</th>
<th>ID</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>E [1/5]</td>
<td>120 ± 33</td>
<td>170 ± 54</td>
<td>286 ± 47</td>
<td>E [1/5]</td>
<td>100 ± 33</td>
<td>286 ± 47</td>
<td>286 ± 47</td>
<td>1.896 ± 383***</td>
<td></td>
</tr>
</tbody>
</table>

Values are means ± S.E.M. from 9 to 3 animals; data were analyzed with ANOVA.

| Values are means from 4 to 6 experiments; data were analyzed with ANOVA. |
| Values are means from 4 to 6 experiments; data were analyzed with ANOVA. |

**Significantly different (P < 0.01) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
**Significantly different (P < 0.001) when pregnant animals are compared with nonpregnant, nonlactating animals. |
insulin and dexamethasone, implying that at least some of the effects observed with these hormones are due to an altered activity of the promoter (Travers and Barber, unpublished observation).

Lactation results in a readjustment of lipid metabolism to meet the demands of the mammary gland for milk fat synthesis, and this is associated with an increase in both the activity and amount of mammary ACC (Mackall and Lane, 1977) and a corresponding change in the abundance of ACC-α mRNA (Lopez-Casillas et al., 1991). There are no PI transcripts expressed in mammary tissue; hence, the increase observed seems to be due to an increase in transcripts from PII (and as described later, from PIII). This has been shown to be the case in sheep and rats (Lopez-Casillas et al., 1991; Barber and Travers, 1998). However, because this promoter is constitutively active in all other tissues, it must contain mammary-specific enhancer elements that, due to its activation in lactation, possibly are responsive to lactogenic hormone combinations (e.g., prolactin, possibly in conjunction with insulin and/or glucocorticoids). The involvement of prolactin in the regulation of this promoter is supported by some work with rats. In that study lactating rats either had their litters removed or were given bromocryptine to reduce their serum prolactin levels, and this resulted in a 5- to 10-fold decrease in the level of ACC-α transcripts. Concurrent administration of prolactin with the bromocryptine prevented this decrease (Barber et al., 1992).

Some of the rat PII sequence has been published (GenBank accession no. X51980), and comparison of this with that of the ovine sequence (GenBank accession no. AJ292285) showed that this promoter lacks TATA and CCAAT boxes in both of these species, elements which are commonly absent from constitutively active promoters (Kim and Tae, 1994). A number of consensus sites for transcription factor binding have been identified in this promoter, and in some cases the factors they bind and their involvement in a particular response have been elucidated; for example, two sites that bind the factor Sp1 have been shown to be involved in the activation of this promoter by glucose in 30A5 preadipocytes (Daniel et al., 1996). As yet no work has identified sites that may be involved in the mammary-specific induction of this promoter.

**ACC-α E5A-Type Transcript**

A novel ACC-α transcript type has been identified in ovine mammary gland. This variant transcript contained a 5′ leader sequence that consisted of a long 5′ UTR and a 17-amino-acid open reading frame. Translation of this transcript would result in an ACC-α in which the 76-amino-acid N-terminal sequence encoded by exon 5 would be replaced by this 17-amino-acid sequence, the initiator methionine of which was preceded by several upstream in-frame stop codons. Because this transcript differed from those previously identified at the junction of exon 6, and the novel transcript was thought to represent the alternative splicing of one or more exons prior to exon 6, it was termed the E5A-type transcript. When the sequence was localized in genomic DNA it was found to be represented by a single exon between exons 5 and 6 (Figure 2) (Barber and Travers, 1998).

The tissue distribution of the E5A-type transcript was determined using an RNase protection assay, which showed that expression of this transcript was tissue-restricted, being undetectable in adipose tissue, heart, and skeletal muscle but present in liver, kidney, lung, and brain (not shown) and at very high levels in lactating mammary gland (Figure 3).

The high level of expression in lactating mammary gland prompted an investigation of the regulation of the transcript in pregnancy and lactation, and the abundance of E5A-type transcripts was found to increase markedly from control to 18-d lactating mammary tissue. When the DNA content of the tissue was taken into account ACC-α transcripts containing exon 5 increased approximately threefold from nonpregnant to lactating tissue, compared to an almost 15-fold increase in E5A-type transcripts. The E5A-type transcript represents only a few percentage units of total ACC-α mRNA in control tissue, and even with this large increase it remained the minor transcript in lactation, making up almost one-third of the total ACC-α mRNA (Figure 4). It is worth noting that although transcripts containing the E5A sequence were successfully translated in vitro to give a protein fragment of the expected size, the sequence context around the predicted initiation methionine corresponds to a suboptimal Kozak initiation sequence when compared with that in exon 5 (Kozak, 1986), suggesting there could be differences in the translatability of these transcripts.

Because the 5′ end of the transcript corresponded to the 5′ extent of the E5A exon (i.e., it seemed to represent a primary coding exon), a sequence of approximately 1.5 kb adjacent to the exon was cloned into a luciferase reporter vector to determine whether this demonstrated promoter activity. This construct was transfected into HepG2 and HC11 cells, human hepatoma, and mouse mammary epithelial cell lines, respectively; in both cell types it functioned as a promoter and its activity was stimulated by insulin. This promoter was termed Promoter III (PIII) (Barber and Travers, 1998). The physiological significance of an insulin response is uncertain, particularly in a promoter that demonstrates an increased activity in mammary gland during lactation, when serum insulin levels are decreased relative to control. Burnol et al. (1986) have, however, produced some evidence that the mammary gland at this time may have an increased sensitivity to insulin so this may remain a potentially physiologically significant response.

Initial examination of the sequence of PIII showed that it lacks both TATA and CCAAT boxes proximal to the transcription start site, having instead a sequence...
Acetyl-CoA carboxylase-α homologous to the inr sequence CTCANTCT, found in a large number of TATA-less promoters (Smale, 1997). In addition, consensus sequences for binding of a number of other transcription factors, including an E-box and an inverted CCAAT box element (ICE), were found.

In addition, consensus sequences for binding of a number of other transcription factors, including an E-box and an inverted CCAAT box element (ICE), were found.

Figure 3. Expression of PIII ACC-α transcripts in tissues from sheep by RNase protection assay. CMG = nonpregnant, nonlactating mammary gland; LMG = lactating mammary gland.

Figure 4. Expression of PII and PIII ACC-α transcripts in ovine mammary gland during pregnancy and lactation (redrawn from data in Barber and Travers, 1998).

but their functional significance remains to be determined.

The E5A isozyme diverges from normal ACC-α at the N-terminus, which is also the point at which the ACC-β is most different (Ha et al., 1996; Abu-Elheiga et al., 1997). In ACC-β this results in a hydrophobic terminal sequence thought to target this isozyme to the mitochondrial membrane, whereas in the E5A sequence the N-terminus is predicted to be a short hydrophilic helix, suggesting it may have a function other than targeting.

Phosphorylation at Ser79 and Ser1200 in rat ACC-α affects enzyme activity, and these residues are targets for AMP-activated protein kinase and cAMP-activated-protein kinase respectively, at least in vitro (Ha et al., 1994). The consensus sequence for phosphorylation at Ser79 (Ser80 in the ovine sequence) spans the boundary with exon 5, and substitution of the E5A sequence, therefore, disrupts this, the methionine being replaced by a glutamine in E5A (Figure 5). Hardie and coworkers have shown using in vitro assays with synthesized peptide that a substitution of this type may alter the susceptibility of the molecule to phosphorylation (Dale et al., 1995; Ching et al., 1996), and extrapolation from these in vitro experiments suggests at least the possibility of an ACC-α isozyme in mammary gland that could be more resistant to phosphorylation and therefore inactivation.

Implications

A major feature of the metabolic adaptation that results in transfer of lipogenic capacity through repression and activation of the acetyl-CoA carboxylase-α (ACC-α) gene in adipose tissue and mammary gland, respectively, during lactation is that it is largely promoter (P)-specific. The PI is potently repressed in adipocytes, PII activity is only modulated slightly, and corresponding changes in mammary gland involve modest induction of PII and potent induction of PIII. Transcription from PI and PIII, respectively, seem necessary for
the differentiative function of the adipocyte and mammary epithelial cell, respectively. The ACC-α transcript heterogeneity may be involved in the segregation of metabolic pathways into discrete cellular compartments, or in the case of the E5A transcript, in which the protein sequence is altered, an effect on enzyme kinetics may also be inferred. Further research will be required to work out the molecular details of this speculation.

Literature Cited


