REGULATION OF MAMMALIAN ACETYL-COENZYME A CARBOXYLASE

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ABSTRACT

Long-chain fatty acids are involved in all aspects of cellular structure and function. For controlling amounts of fatty acids, cells are endowed with two acetyl-coenzyme A carboxylase (ACC) systems. ACC-α is the rate-limiting enzyme in the biogenesis of long-chain fatty acids, and ACC-β is believed to control mitochondrial fatty acid oxidation. These two isoforms of ACC control the amount of fatty acids in the cells. Phosphorylation and dephosphorylation of ACC-α cause enzyme inactivation and activation, respectively, and serve as the enzyme's short-term regulatory mechanism. Covalently modified enzymes become more sensitive toward cellular metabolites. In addition, many hormones and nutrients affect gene expression. The gene products formed are heterogeneous and tissue specific. The ACC-β gene is located on human chromosome 12; the cDNA for this gene has just been cloned. The gene for the α-isoform is located on human chromosome 17. The catalytic core of the β-isoform is homologous to that of the α-isoform, except for an additional peptide of about 150 amino acids at the N terminus. This extra peptide sequence makes the β-form about 10,000 daltons larger, and it is thought to be involved in the unique role that has been assigned to this enzyme. The detailed control mechanisms for the β-isoform are not known.

CONTENTS

INTRODUCTION

Long-chain fatty acids and their derivatives are not only constituents of cellular structures, they also function as regulatory molecules, affecting all phases of cellular activities. They are the main energy reserve and the primary component of membranes, as well as of such entities as lung surfactant. Fatty acylation of some regulatory proteins affects their localization, trafficking, and function. Also, as components of lipid second messengers, they perform indispensable regulatory functions by mediating the signal transduction of various external and internal agents.

Acetyl-coenzyme A (Co-A) carboxylase (ACC) (EC 6.4.1.2) is one of the two enzymes required for long-chain fatty acid synthesis; the other is fatty acid synthase (EC 2.3.1.85). The reactions catalyzed by these enzymes are shown in Figure 1. ACC catalyzes the carboxylation of acetyl-CoA in the generation of malonyl-CoA (reactions 1 and 2). Malonyl-CoA is then used for long-chain fatty acid synthesis by the multifunctional enzyme, fatty acid synthase (reaction 3), which involves seven different enzyme reactions for the synthesis of palmitic acid.

In addition to being an intermediate in the synthesis of long-chain fatty acids, malonyl-CoA, the reaction product of ACC, may serve as a key regulatory agent in the oxidation of fatty acids by controlling the activity of carnitine-palmitoyl CoA transferase 1 (CPT-I) (EC 2.3.1.7) at the mitochondrial transport step of long-chain fatty acid oxidation (62, 63). The unique involvement of ACC in both fatty acid synthesis and degradation, and the complex roles that fatty acids with different half-lives play as cellular components, suggest that ACC activity must be precisely controlled. In addition, the position of ACC in the metabolic
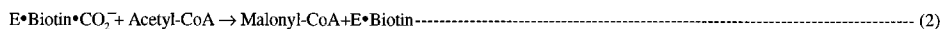
$$HCO_3^- + ATP + E \bullet Biotin \rightarrow E \bullet Biotin \bullet CO_2^- + ADP + Pi \tag{1}$$

$$E \bullet Biotin \bullet CO_2^- + Acetyl\text{-}CoA \rightarrow Malonyl\text{-}CoA + E \bullet Biotin \tag{2}$$

$$Acetyl\text{-}CoA + 7\,Malonyl\text{-}CoA + 14\,NADPH + 14H^+ + 7ATP + H_2O \rightarrow palmitic\ acid + 8\,CoA + 14\,NADP^+ + 7ADP + 7Pi \tag{3}$$
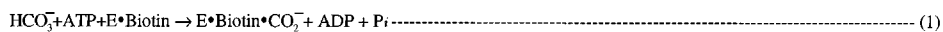
Figure 1   Reactions catalyzed by acetyl-coenzyme A carboxylase and fatty acid synthase.

pathway demands complex and interlocking regulatory mechanisms for this enzyme.

This review discusses some of the salient features of the regulatory mechanisms for mammalian ACC. The short-term regulatory mechanisms for ACC, such as the allosteric control mechanism involving cellular metabolites and reversible covalent modification of ACC, have been reviewed previously (31, 38, 39, 45, 97, 98). Therefore, the long-term regulatory mechanisms, particularly those acting at the gene level, are emphasized in this review. This review focuses on unresolved questions and recent developments. My laboratory has been the primary contributor to investigations of the regulation of ACC at the gene level. Therefore, the review of this aspect of ACC is based primarily on work from my laboratory. Two forms of ACC have now been identified. The enzyme that is involved in the synthesis of long-chain fatty acids and has a molecular weight of 265,000 has been designated ACC-α. The isoform with a molecular weight of 275,000–280,000 has been called ACC-β; it may be involved in the regulation of mitochondrial oxidation of fatty acids (100).

ROLE OF ACC

ACC as the Rate-Limiting Enzyme in the Biogenesis of Fatty Acids

Both the activity of ACC and the rate of fatty acid synthesis fluctuate rapidly in response to various internal and external factors that affect lipogenesis, such as hormonal (22, 33, 46, 58, 59, 96, 103, 104), dietary (13, 23, 41, 55, 68, 70, 74), developmental (7, 17, 55, 86), and genetic factors (12, 70, 73). These environmental factors exert their effects at both the protein level and the gene level. Based on studies conducted in vitro, however, the relative catalytic capacity of ACC is very low compared with that of fatty acid synthase in the synthesis of long-chain fatty acids (69), which suggests that ACC activity is the rate-limiting factor in determining the rate of fatty acid synthesis. The rate-limiting nature of ACC has been more clearly demonstrated in a recent report where the specific destruction of ACC-α mRNA was carried out by expressing an ACC mRNA-specific ribozyme gene. The rate of fatty acid synthesis in that system was shown to be commensurate with the amount of ACC mRNA and ACC protein (28).

ACC as the Regulator of Fatty Acid Oxidation

Fatty acid oxidation in mammalian systems is carried out by peroxisomes and mitochondria. Peroxisomal fatty acid oxidation occurs following fatty acid activation to fatty acyl-CoA by acyl-CoA synthase at the peroxisomal membrane (61); fatty acid uptake, in this system, does not involve carnitine and is not regulated (89). In contrast, mitochondrial fatty acid oxidation involves activation

of fatty acid to fatty acyl-CoA, which is then taken into the mitochondria via the CPT system (62, 63). The CPT-I at the mitochondrial membrane is extremely sensitive to inhibition by malonyl-CoA; the K_i of malonyl-CoA is in the micromolar range. Thus, malonyl-CoA and ACC are thought to be physiological regulators of mitochondrial fatty acid oxidation (62, 63).

The general belief that ACC plays a role in the control of fatty acid oxidation was based on the following observations. First, CPT-I, an essential component of mitochondrial fatty acid oxidation, is extremely sensitive to inhibition by malonyl-CoA and malonyl-CoA is only generated by ACC. Second, skeletal and heart muscle are tissues that use long-chain fatty acids for energy sources but that are nonlipogenic; they contain a large amount of ACC (87, 93). The ACC in these mitochondria-rich tissues is mostly ACC-β, which has a molecular weight of 275,000–280,000 (8, 78, 94, 102). Physiological conditions that cause a decrease in ACC activity and malonyl-CoA levels in muscle tissues are accompanied by accelerated rates of fatty acid oxidation in the liver and heart (4, 49, 78, 99, 101), which suggests a possible causal relationship between ACC activity and the rate of fatty acid oxidation.

The malonyl-CoA binding component of CPT-I faces the outer phase (the cytosolic sphere) of the outer membrane of the mitochondrion (60), whereas the catalytic region of CPT-I faces the inner phase of the outer membrane. It follows, therefore, that control of the concentration of malonyl-CoA near the malonyl-CoA binding component of CPT-I is a vital aspect of the regulation of fatty acid oxidation.

The new isoform of ACC, ACC-β, has been purified (87, 94), and its gene has been cloned (29, 100). ACC-β has been implicated as the ACC that generates malonyl-CoA and controls fatty acid oxidation (4, 49, 78, 99, 101). However, a clear division of labor between ACC-α and ACC-β in fatty acid synthesis and oxidation, respectively, has not been defined experimentally. Indeed, fatty acid oxidation is increased when an ACC-α mRNA–specific anti-sense gene is expressed in INS-1 cells, indicating that ACC-α also may be involved in mitochondrial fatty acid oxidation (S Zhang & K-H Kim, unpublished data). In addition, mitochondrial fatty acid oxidation may be controlled by changes in the sensitivity of CPT-I toward malonyl-CoA under certain physiological conditions (14, 26, 30, 76).

REGULATION OF ACC BY COVALENT MODIFICATION

Both ACC-α and ACC-β are phosphoproteins. Since the original report that ACC activity was inactivated by phosphorylation of the protein (10, 11), extensive efforts have been made to clarify the mechanism of this covalent modification. However, many of these experiments were done without any knowledge

of the existence of ACC-β. There is some evidence that ACC-β may be phosphorylated under in vivo and in vitro conditions (99, 102), although the effect of phosphorylation on ACC-β activity has yet to be examined. This causes some uncertainty in reviewing earlier experimental results, because the correlation between phosphate incorporation and enzyme inactivation was analyzed without considering possible differences between ACC-α and ACC-β. However, the control of ACC-α activity by covalent phosphorylation is well established, and this topic is discussed below.

ACC-α Regulation by Reversible Phosphorylation

Carefully prepared ACC contains multiple phosphates: Both the rat liver and mammary gland enzymes contain as many as 9 mol of phosphate/mole of enzyme subunit (83, 88, 106). These numbers represent total phosphates in the protein and include phosphate on sites that affect enzyme activity as well as on sites that do not affect enzyme activity directly. Furthermore, when these studies were done, the occurrence of ACC-β was not known. Thus, the significance of the number of phosphates per mole of enzyme requires additional careful investigation. Nevertheless, based on sequence analysis of the phosphopeptides of ACC-α, there are at least eight phosphorylation sites on ACC-α. These are clustered in an interesting manner: Six of the eight phosphorylation sites are localized on the first 100 amino acid residues at the N terminus (Figure 2). The remaining sites are Ser-1200 and Ser-1215. Phosphorylation by the AMP-activated protein kinase causes a large decrease in V_{max} (67). Phosphorylation
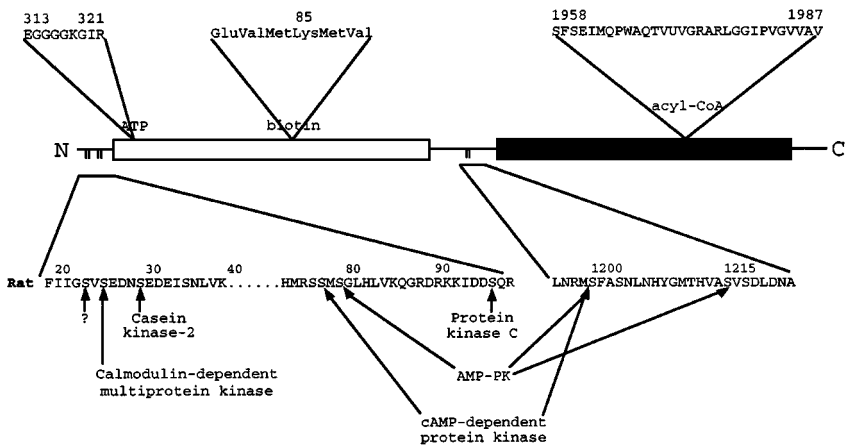

Figure 2 Domain map of acetyl-coenzyme A carboxylase showing locations of functional and phosphorylation sites.

by cAMP-dependent protein kinase and ACC kinase (67) causes primarily an increase in the K_a for citrate and a more modest decrease in V_{max} (67).

The isolation and characterization of the various phosphopeptides of ACC-α that can be phosphorylated by different protein kinases in vitro and in vivo (32, 35, 67) made it possible to identify the phosphorylation sites for specific protein kinases that control ACC activity under different physiological conditions. Rat liver ACC-α is coded by 7,035 nucleotides, which encode 2,345 amino acids, making it 265,220 Da in size (50). Alignment of the phosphopeptide sequences with the amino acid sequence of ACC-α deduced from the nucleotide sequence of rat cDNA allowed identification of the specific phosphorylation sites of ACC-α that are affected by different protein kinases.

Eight different phosphorylation sites on ACC-α have been identified: serine 23, 25, 29, 77, 79, 95, 1200, and 1215 (Figure 2). Among the identified protein kinases, only cAMP-dependent protein kinase and 5′-AMP–dependent protein kinase can inactivate ACC in vitro. cAMP-dependent protein kinase inactivates the enzyme by phosphorylation of Ser-77 and -1200, whereas 5′-AMP–dependent protein kinase phosphorylates Ser-79, -1200, and -1215. These two protein kinases phosphorylate these multiple sites simultaneously, and it was not immediately apparent which site(s) was the critical one for enzyme inactivation.

In an attempt to identify the critical phosphorylation site(s), ACC-α cDNA containing the entire coding region was constructed and expressed at a high level by means of the T7 RNA polymerase/promoter expression system (21). Properties of the expressed ACC were the same as those of the endogenous ACC with respect to molecular weight, cross-reactivity with the anti-ACC antiserum, and inactivation pattern by 5′-AMP–dependent protein kinase and cAMP-dependent protein kinase (27). Site-specific mutations were introduced at serine 77, 79, 1200, and 1215. Analysis of these mutated ACCs showed that serine 1200 was critical for inactivation of ACC by cAMP-dependent protein kinase and that serine 79 was critical for inactivation of ACC by 5′-AMP–dependent protein kinase (27). Use of the expressed ACC-α protein in these phosphorylation studies clearly established the occurrence of the covalent modification mechanism in the ACC-α system, because, in these experiments, no ACC-β was present to complicate the results.

However, many questions remain. There are suggestions that the site of action of cAMP-dependent protein kinase is not ACC, but rather that the site is perhaps ACC-specific cAMP-independent protein kinase (47) or protein phosphatase (31). These reports suggest that cAMP is involved at multiple sites whose phosphorylation collectively or independently leads to the inactivation of ACC. Such multi-site effects are not uncommon in metabolic control systems.

The roles of other phosphorylation sites (other than Ser-79 and Ser-1200) in the control of ACC activity or function are not known. For example, how

phosphorylation of these "silent" sites affects phosphorylation of other sites, including those identified as critical, as in the case of the glycogen synthase system (19, 20), has yet to be examined. The possibility of a role for the silent phosphorylation site has been suggested by the observation that when Ser-79 is mutated, Ser-1200 was not phosphorylated by 5′-AMP–dependent protein kinase, in spite of its ability to phosphorylate Ser-1200 in the native enzyme (27). Thus, phosphorylation of Ser-1200 may require prior phosphorylation of Ser-79. On the other hand, the inability of AMP-dependent protein kinase to phosphorylate Ser-1200 may be related not to phosphorylation of Ser-79 but to a secondary effect of the mutation.

Ser-96 is phosphorylated by protein kinase C. Phosphorylation of ACC by protein kinase C may result in enzyme activation, and this site may be the one whose phosphorylation is stimulated by insulin (95). However, although insulin activates ACC, whether or not this is due to ACC phosphorylation is uncertain. Insulin activation of ACC apparently involves the phosphorylation of a low-molecular-weight effector and not ACC itself (34). Phorbol esters mimic the stimulation of phosphorylation caused by insulin (35) but do not activate ACC. In our hands, protein kinase C phosphorylates purified ACC in vitro, and this is accompanied by a small inactivation of ACC. These studies collectively suggest that Ser-96, the site phosphorylated by protein kinase C, is not the site regulated by insulin. Although insulin activates ACC and stimulates its phosphorylation, a direct causal relationship between these events has not been established.

In 3T3-L1 cells, the activity of casein kinase II is stimulated by insulin (105), suggesting that insulin may promote phosphorylation of ACC through casein kinase II. Casein kinase II phosphorylated ACC at Ser-29, but enzyme activity was not affected (35, 90). Phosphorylation of ACC by casein kinase II may promote dephosphorylation of those sites that affect enzyme activity (82). Insulin does promote phosphorylation of ACC (36, 105); however, there is no direct evidence that insulin-promoted phosphorylation causes insulin-promoted enzyme activation. Insulin treatment of fat cells increased association of protein phosphatase with ACC (44), which suggests that insulin activation of ACC may be due to dephosphorylation.

Ser-25 is phosphorylated by calmodulin-dependent protein kinase, but this does not lead to inactivation. Ser-25, however, is near Ser-29, which is phosphorylated by casein kinase II, providing the interesting structural feature -Ser(P)-X-X-X-Ser(P)-. In glycogen synthase and in the G component of protein phosphatase I (EC 3.1.3.16), phosphorylation of a serine residue (toward the COOH terminus) by casein kinase II creates a structural feature that is necessary for recognition by other kinases and, thus, initiates phosphorylation of the other sites at the N-terminal side (19, 20). Thus, phosphorylation of

Ser-29 of ACC by casein kinase II may allow Ser-25 to be phosphorylated by a calmodulin-dependent protein kinase. Indeed, ACC lacking phosphate at Ser-29 is a poor substrate for calmodulin-dependent protein kinase. Thus, it would be interesting to examine the effect of prior phosphorylation by casein kinase II on calmodulin-dependent protein kinase action and the effect of such phosphorylation on the activity.

GENES AND STRUCTURE OF ACC

Both ACC-α (1, 5, 50, 92) and ACC-β (29, 100) cDNA have been cloned from different mammalian sources. The ACC-α gene is located on human chromosome 17 (1, 66) and the ACC-β gene is on human chromosome 12 (100). Details of the gene structures of these isoforms are not yet known, except for that of the promoter of ACC-α, which is discussed in the following section. In this section, the primary structures of ACC-α and ACC-β are contrasted (Figure 3). Human ACC-α consists of 2,346 amino acids with a molecular weight of 264,737 (1), whereas ACC-β from humans contains 2,458 amino acids with a molecular weight of 276,638 (29). Amino acid sequences of the three functional sites—the ATP binding site, carboxylation site (biotin binding site), and acyl-CoA binding site, as well as the critical phosphorylation sites— are virtually identical in both forms. ACC-β contains about 150 extra amino acids at the N terminus, and the positions of these functional sites are displaced by about the same number of amino acids in the β-form. The sequences of the

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                    MVLLLCLSRLIFSCLTFSWLKIWGKMTDSKPITKSKSEANLIPSQEPFPASDNSGETPQR  60
5'-end of           NGEGHTLPKTPSQAEPASHKGPKDAGRRRNSLPPSHQKPPRNPLSSSDAAHSPELQANGT  120
ACC-β               GTQGLEVTDTNGLSSSARPQGQQAGSPSKEDKKQANIKRQLMTNFILGSFDDYSSDEDSV  180
                    AGSSRESTRKGSRASLGALS
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Biotin-site   α: (884) E V M Ⓚ M (889)
               β: (927) E V M Ⓚ M (931)

ATP-site   α: (307) E G G G G K G I (314)
               β: (459) E G G G G K G I (466)

cAMP-PK   α: (1198) N R M Ⓢ F S S (1203)
site   β: (1334) N R M Ⓣ V P L (1340)

5'AMP-PK   α: (75) R S S W Ⓢ G (80)
site   β: (218) R P S M Ⓢ G (224)

Acyl-CoA   α: (1959) S F S E I M O P W A Q T V V V G R A R L G G I P V G V V A V(1988)
binding site   β: (2071) S F K E I M A P W A Q T V V T G R A R L G G I P V G V I A V(2100)

Figure 3   Comparison of the sequences of acetyl-coenzyme A carboxylase (ACC)-α and ACC-β sequences around the functional and phosphorylation sites.

remaining amino acid regions of the two forms of ACC are about 85% similar, with 75% identity. The extra N terminus of the β-form may be related to the specific function that this isoform performs in the control of mitochondrial fatty acid uptake and oxidation in mitochondria.

The unique N-terminal sequence of ACC-β is rich in hydroxy amino acids (46 serines and threonines) and basic amino acids (25 arginines and lysines). Such an amino acid composition is typical for transit peptides that are targeted to mitochondria and chloroplasts (64). However, the ACC-β sequence is interrupted by 17 proline residues between residue 31 and 147. Although there is an abundance of basic amino acids, they are counterbalanced by a comparable number of acidic residues (23 glutamic and aspartic acids). Not only is the whole sequence comparatively long to be a signal peptide, the above features would make it difficult to form the amphiphilical secondary structures that are found in the mitochondrial import proteins. Overall, the unique sequence exhibits strong hydrophilic features, except for the first 25–amino acid portion of the N terminus, which contains a hydrophobic region surrounded by positive charges.

One hypothesis is that this region of ACC-β is required for targeting and/or anchoring to the mitochondrial outer membrane (Figure 4). The hydrophobic region (the first 25 amino acids) may bind or anchor ACC-β into the outer membrane of the mitochondrion. This could control CPT-I activity by generating malonyl-CoA at or near the malonyl-CoA binding site of CPT-I (60). A second hypothesis concerning this model is that the phosphorylation of the mito-sequence at serine and threonine residues inhibits binding or anchoring of ACC-β to the membrane by increasing negative charges on the mito-sequence. In the absence of phosphorylation of a large number of hydroxy amino acids, the basic amino acids on the mito-sequence would interact with the negative charges of the membrane lipids and augment the hydrophobic interaction between the hydrophobic region of ACC-β and the outer membrane. Such interactions might increase the local concentration of a metabolite as much as 1000-fold (80).

As much as 75% of some forms of ACC appear to bind reversibly to the outer membrane of liver mitochondria. The degree of binding depends on the physiological condition of the donor rats (2, 77). However, the association of ACC-β with mitochondria has been difficult to demonstrate (18, 37). The reasons for the differences in the conclusions from these two studies are not clear. However, the experimental designs used in these studies were not the same. Furthermore, it is possible that proteolytic cleavage of the N-terminal segment occurred during subcellular fractionation, releasing the catalytic domain of ACC-β to the cytosol.
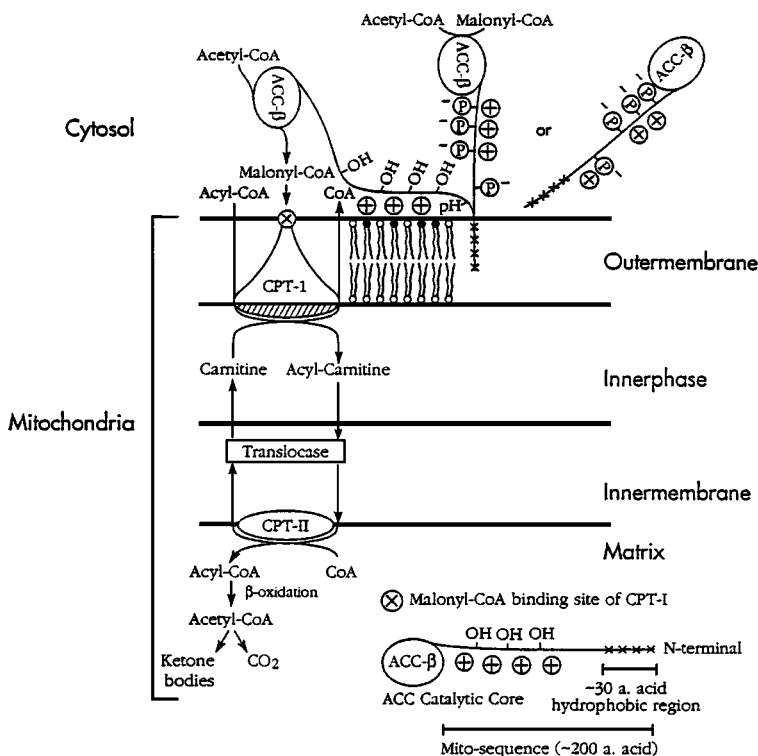
Figure 4 Role of acetyl-coenzyme A (CoA) carboxylase (ACC)-β in the control of carnitine palmitoyl-CoA transferase I (CPT-I). In this model, the N-terminal hydrophobic region of the mito-sequence is responsible for anchoring or binding ACC-β in such a way as to control malonyl-CoA concentration near the malonyl-CoA binding site of CPT-I. Phosphorylation-dephosphorylation of the hydroxy amino acid residues in the mito-sequence may control the binding or anchoring of ACC-β to the outer membrane of the mitochondrion. (From Reference 29.)

PROMOTER STRUCTURE OF ACC-α

Heterogeneous Gene Products and Their Physiological Role

Various techniques have been used to identify five forms of ACC mRNA (43, 51, 57). The occurrence of a variety of ACC-α mRNAs is the result of the functioning of two independent promoters (noted as PI and PII in Figure 5) and differential splicing of the primary transcripts (Figure 5). Class 1 mRNAs are transcribed from PI and have exon 1 at their 5′ ends. Class 2 mRNAs are transcribed from PII and have exon 2 at their 5′ ends. The major ACC-α mRNAs transcribed from PI contain a 5′-untranslated region with exons 1, 4,
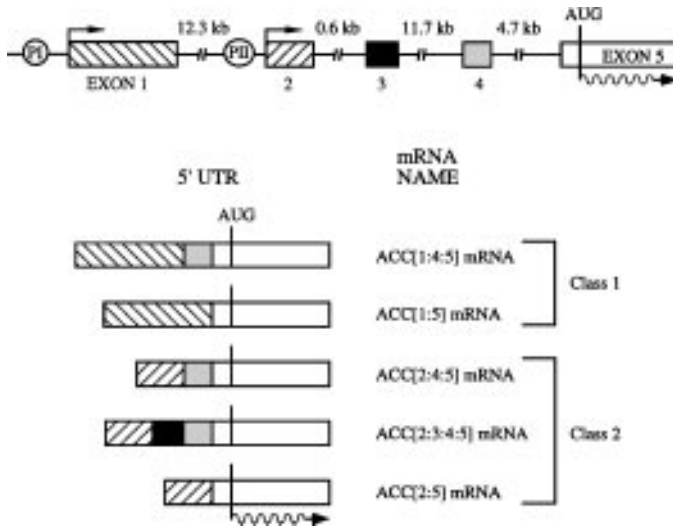
Figure 5 Structure of the 5′ end of the rat acetyl-coenzyme A carboxylase (ACC)-α gene and of the different classes of rat ACC mRNA. The upper panel shows a genomic map of the first five exons of the ACC gene (*rectangular boxes*); sizes and relative positions are indicated. The sizes and positions of the intervening sequences (IVS) separating those exons are also shown. The positions of the two ACC gene promoters are indicated by the circles labeled PI and PII. Transcription start sites are indicated by right-angled arrows above the boxes for exon 1 and 2. The opening AUG codon of the open reading frame of ACC is indicated by a solid vertical line across the exon 5 box. The coding regions of the ACC mRNA open reading frame are symbolized by a wavy arrow. The lower panel depicts the 5′-end structures of the different types of ACC mRNA identified to date and their division into two distinct classes based on the promoter that generated them. The exons composing the 5′-UTRs of these mRNAs can also be identified from the upper panel by matching the pattern of the rectangular boxes depicting them.

and 5 and is designated ACC[1:4:5]mRNA. The predominant form of ACC mRNA from PII contains exons 2, 4, and 5 in its 5′-untranslated region and is designated ACC[2:4:5]mRNA. The other forms of mRNA contain different combinations of exons and are minor species. The different ACC mRNAs are expressed tissue specifically, depending on the physiological state of the tissue (6, 42, 43, 51, 53, 57, 75, 84, 107). The physiological significance of the different forms of this mRNA, with all forms containing the same coding sequence, is not known. However, the generation of multiple mRNAs may provide an additional level of ACC regulation at the gene level and also at the translational level. The 5′-untranslated regions do affect translational efficiency in vitro (52), raising the possibility that the 5′-untranslated regions are involved in the regulation of translation of specific mRNA species.

The abundances of the different mRNAs under different physiological conditions was examined to gain some insight into the physiological significance of the multiple forms of ACC mRNA. Conventional analytical methods, such as dot blot or Northern (RNA) blot analysis using cDNA fragments from the coding regions of the mRNA, measure only the total amount of ACC mRNA. Such an approach reflects the patterns of the total ACC mRNA metabolism and not the function of the two promoters or alternative splicing. Unfortunately, with the exception of a few cases (6, 42, 52, 53, 75, 84, 107), most measurements of ACC mRNA abundance have used conventional analytical methods.

In a primer extension analysis, transcripts that start at different promoters or that have alternative splicing are distinguished by the sizes of the primer extension products (Figure 5). This allows the expression of each form of ACC mRNA to be measured quantitatively (75) and provided evidence about the activities of the two promoters under different physiological conditions. The primer must be complementary to the coding region at its 5′-end, a region shared by all ACC-α mRNAs. This approach also precludes complications due to the presence of ACC-β, because its 5′-end is different from that of ACC-α.

ACC-α gene activity in the mammary gland, liver, and white adipose tissue during pregnancy and lactation was examined (Figure 6). Seven days before parturition, ACC gene products were almost undetectable. Just before parturition, the exocrine component of the mammary glands is activated and ACC[2:4:5]mRNA appears, indicating the activation of PII. The activity of PII increases strikingly upon parturition, reaching a peak at day +1 and remaining high until day +4. This increased activity is manifested by the presence of both ACC[2:4:5] and ACC[2:3:4:5]mRNA, although the amounts of the latter are rather small compared with the former. Even though the amount of ACC[2:3:4:5]mRNA produced in the lactating mammary gland is small, it is the only tissue that contains detectable amounts of this ACC mRNA. Importantly, despite a 30- to 40-fold increase in ACC enzyme activity in the lactating gland, no PI activity is detectable. During pregnancy, mammary glands contain a significant amount of fat tissue, and yet no PI activity, the hallmark of ACC gene activity in adipose tissue, can be detected. Feeding a fat-free diet from day 7 before parturition through day 7 of the lactation period failed to activate PI in the mammary gland. This indicates that, in contrast to epididymal white adipose tissue (40, 75) and liver (40, 51, 54, 75), ACC gene activity in the mammary gland is restricted to the PII promoter.

Hepatic ACC gene activities also were measured during the pregnancy-lactation period (Figure 6). Only the PII promoter was active. The small amounts of PII products in the livers of pregnant animals decrease somewhat
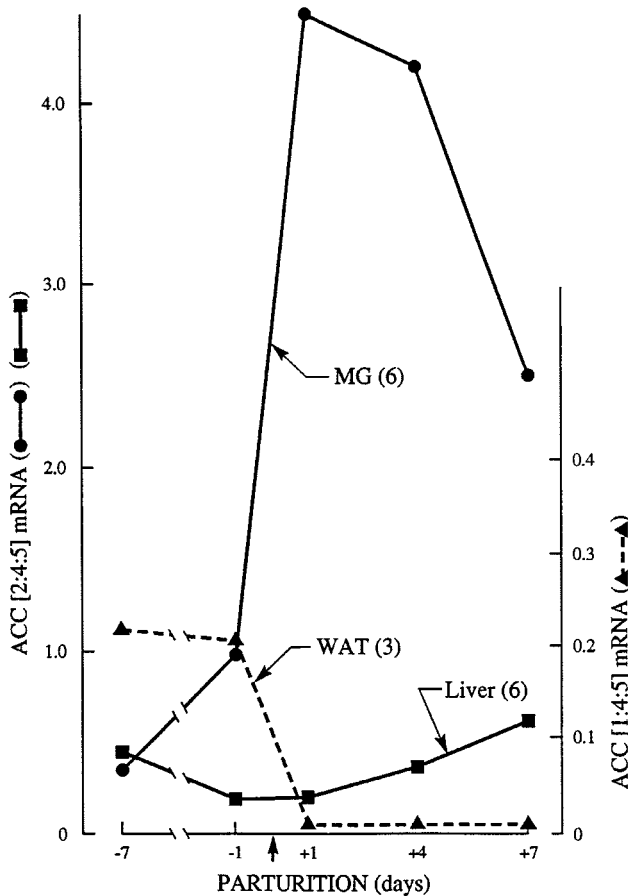
Figure 6 Expression of different forms of acetyl-coenzyme A carboxylase (ACC)-α mRNA in different tissues during pregnancy and lactation. MG, Mammary gland; WAT, white adipose tissues. The primer extension products were separated by denaturing gel electrophoresis and quantitated by densitometry.

during pregnancy. After parturition, a minor increase in PII activity, as detected by the appearance of ACC[2:4:5]mRNA, is observed. In contrast to the increased hepatic ACC gene activity induced by starvation-refeeding treatment (51), not only did the PI remain silent all through lactation, the observed changes in PII were insignificant. This observation also suggests that lipogenesis carried out in the liver during lactation does not contribute substantially to the fatty acid content of the milk.

ACC gene activity in parametrial white adipose tissue during pregnancy and lactation produces only ACC mRNAs belonging to class 1; this finding is similar to that observed in epididymal fat tissue. However, seven days before parturition, the level of these ACC mRNAs in the white adipose tissue of these female rats is significantly lower than that observed in male adipose tissue. This low level of PI activity diminishes to undetectable levels by the time of parturition and remains shut off through the lactation period. Thus, fatty acids in milk are not imported from the liver or adipose tissue but are primarily synthesized in the mammary gland. Although these changes are regulated by circulating hormonal signals, they produce tissue-specific responses that selectively affect either the PI or PII promoters of the ACC gene in liver, adipose tissue, and mammary gland.

The triglyceride fatty acids that constitute the huge mass of adipose tissue of the genetically obese Zucker rat are mainly synthesized de novo in the liver (3). Analysis of hepatic RNA prepared from littermates of Zucker rats at two and three months of age indicates that the lean siblings at both ages exhibit a pattern of ACC mRNAs that is qualitatively comparable to the normal Wistar rat pattern. The most prevalent species of ACC mRNA is the ACC[2:4:5]mRNA, and there are negligible amounts of class 1 transcripts. On the other hand, in the homozygous obese siblings, the PI ACC gene promoter is active, and a large amount of the ACC[1:4:5]mRNA, a type not detected in the heterozygous lean siblings, is found (54). Importantly, the PI promoter is active in animals fed a normal diet ad libitum, without subjecting them to conditions that stimulate lipogenesis, e.g. starvation followed by feeding a fat-free diet (51). This constitutive expression of the PI ACC gene promoter is quantitatively similar to that in the liver of Wistar rats that have been starved and refed a fat-free diet (51). The total amount of hepatic ACC mRNA (adding both the PI and PII activities) in the fatty Zucker rat is three- to fourfold higher (at two and three months of age, respectively) than that of the ad libitum fed Wistar rat. The three- to fourfold difference is what a Northern or dot blot analysis would have revealed; a primer extension analysis revealed that class 1 ACC mRNAs are mostly responsible for the increase. These interesting tissue-specific patterns of expression of PI and PII under different physiological conditions have also been investigated using the polymerase chain reaction technique (42, 84, 107).

How Are the Promoters Equipped to Respond to Physiological Stimuli?

Patterns of expression of PI and PII gene products under different physiological conditions indicate that the activity of the PI promoter is induced when lipogenesis is stimulated in liver and adipose tissue. PII gene products are

expressed detectably in all tissues, although their level of expression is also subject to regulation.

The DNA sequences required for PI and PII responses to different activation factors are shown in Figure 7. The PI promoter contains typical TATA and CAAT boxes in the vicinity of the transcription initiation site. In addition, PI contains a region that is necessary for the positively acting insulin-response element (H Tae, K-H Kim, unpublished data) (Figure 7), as well as two powerful negative fatty acyl-CoA–responsive elements (H Tae, K-H Kim, unpublished data) (Figure 7) and intrinsic repressor elements (84) (Figure 7). Because of limited space, one element from each promoter is discussed, as regards its affect on gene activity and the transcriptional factors involved.

INTRINSIC REPRESSOR ELEMENT IN PI AND FACTORS INVOLVED IN DEREPRESSION   Class I ACC mRNAs, generated by PI activity, are abundant in epididymal fat tissues, but only a trace amount is found in the liver under normal physiological conditions (51, 55). However, class I mRNAs are induced in the liver under stimulated lipogenic conditions. For example, this group of mRNAs is expressed at a high level in the livers of animals subjected to lipogenic conditions by being fed a fat-free, carbohydrate-rich diet following a 2- to 3-day fast (51, 55). These observations suggest that PI is an inducible promoter that is involved in the synthesis of ACC mRNA under stimulated lipogenic conditions. Questions that have been posed are: (a) Why is PI normally inactive? and (b) what agent(s) can activate this promoter?

The low activity of the PI promoter is due to 28 repeats of the CA sequence, the intrinsic repressor, located 220 bases upstream of the transcription initiation site (84, 85). The presence of this repeat suppresses about 70% of the basal promoter activity, irrespective of the origin of the promoter, as long as it contains a CAAT box (84). The requirement for the CAAT box to realize the repression caused by the CA repeat is novel because proteins bound to these two regions are interacting to repress the ACC-α gene, and because the CAAT-binding protein activates the repressed gene in a unique manner. Repression by the CA repeat requires the CAAT box. When the CAAT box is deleted, the CA repeat activates expression of the ACC gene (84). When the gene is repressed by the CA repeat, it can be activated by overexpression of C/EBP-α, likely due to binding of C/EBP-α to the CAAT box. Thus, the CAAT sequence and the CA-repeat sequence in the promoter participate in both gene repression and gene activation, depending on the concentration of C/EBP-α. Neither the activation nor the repression of a gene promoter that contains no CAAT box, i.e. PII, is affected by the insertion of the CA repeat. C/EBP is a family of proteins that belongs to a class of the basic region-leucine zipper proteins (bZIP class).
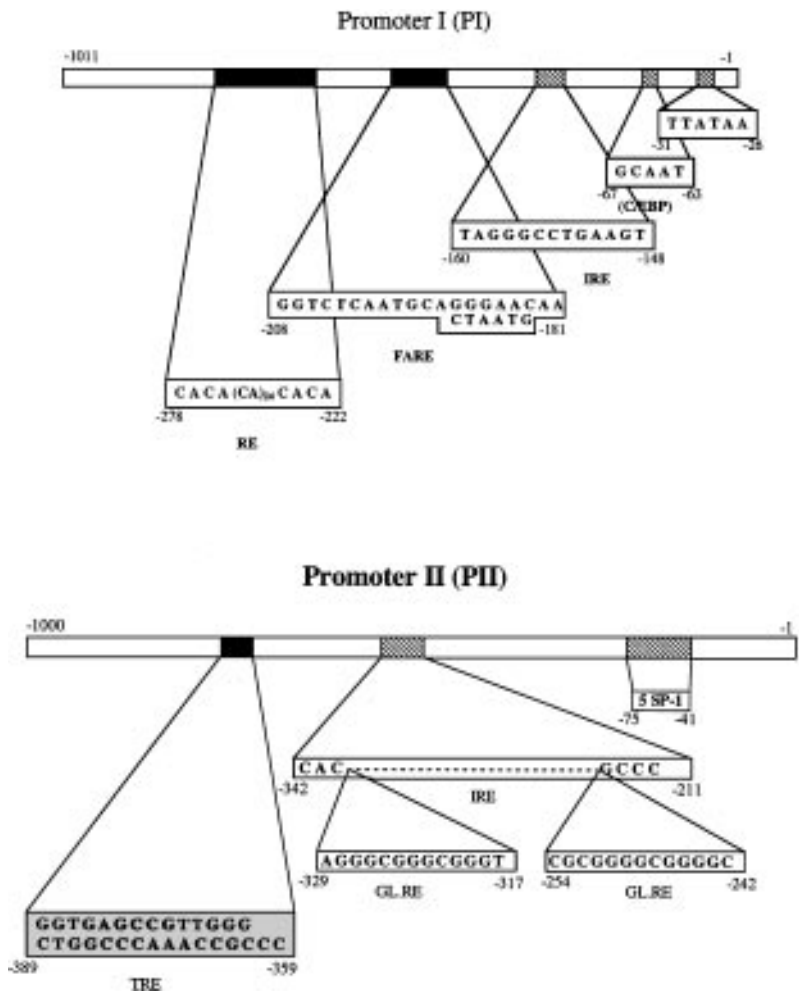
Figure 7   Diagrammatic presentation of ACC-α promoters. Different *cis*-elements in PI (*upper*) and PII (*lower*) are indicated: GCAAT, CAAT box; IRE, insulin-response element; FARE, fatty acyl-coenzyme A–response element; RE, intrinsic receptor element; TRE, tumor necrosis factor– response element; GL.RE, glucose-response element.

C/EBP-β is an isoform of C/EBP-α. Proteins of the C/EBP gene family are capable of forming complexes with other proteins of the family or with proteins not part of the family. The nature of the complexes formed during regulation of specific genes has been difficult to assess. In 30A5 cell cultures, both differentiation and activation of the PI promoter require a short period of cAMP treatment before the cells are exposed to insulin (71). In this case, cAMP activates C/EBP-β gene expression and modifies C/EBP-β by phosphorylation. cAMP does not affect the amount of C/EBP-β binding to the DNA, but it does promote phosphorylation of DNA-bound C/EBP-β and causes PI activation. As in the case of C/EBP-α, C/EBP-β binds to the CAAT box of the PI promoter. These results indicate that cAMP activates PI by inducing C/EBP-β and by activating bound C/EBP-β through phosphorylation; the PI promoter is otherwise repressed by the CA repeat.

GLUCOSE ACTIVATION OF PII; GLUCOSE-RESPONSIVE ELEMENT AND TRANS-CRIPTION FACTOR   The structure of PII is quite different from PI; PII contains neither CAAT nor TATA boxes. However, in spite of PII being a constitutively active promoter, it contains an elaborate array of *cis*-elements that affect its activity (Figure 7, *lower*). The *cis*-elements that have been identified include those for insulin (71), cAMP (71), tumor necrosis factor (72), and glucose-responsive elements (15). In addition, PII contains five SP-1–like sites near the transcription initiation site that serve as powerful enhancers for transcription from the PII promoter (56).

The glucose-response element is used to discuss how this promoter responds to an external stimulator. Lipogenic tissues of eukaryotes utilize glucose as the primary precursor for the synthesis of long-chain fatty acids. Therefore, carbohydrate intake in excess of current caloric requirements causes conversion of excess carbohydrate to triglycerides in the liver. This process is accompanied by the induction of many of the key enzymes of glycolysis, those of fatty acid synthesis, and those involved in triglyceride synthesis (24, 25, 91). Some of the increase in enzyme activity in response to glucose is regulated at the transcriptional level, for example, in the case of L-pyruvate kinase (48), spot 14 (S14) (81), and insulin. A DNA sequence that is required for a glucose/carbohydrate response has been identified on the promoters of these genes. Transcription factors of the MLTF/USF family bind in vitro to carbohydrate-response elements on the L-pyruvate kinase and S14 promoters; these factors appear to function in concert with other tissue-specific factors, such as LFA1 (65).

The DNA sequences in the regions of the glucose-responsive elements in PII and in the L-pyruvate kinase gene are similar. However, in the case of PII the sequence required for glucose induction is very different from that of L-pyruvate kinase. Examination of the expression pattern of a series of deletion

constructs of PII showed that the region from -340 to -249 is essential for ACC induction by glucose (15). Further studies using electrophoretic mobility shift assays, supershift assays, and DNase I footprinting studies established that the binding of the transcription factor Sp1 at the two GC-rich sequences located within the -340 to -249 region of promoter II is important to glucose action (15). Mutations at the GC-rich sequences prevented binding of Sp1, and the induction of the PII promoter was no longer observed. Finally, cotransfection of Sp1 expression and PII-CAT constructs in *Drosophila* Schneider SL2 cells, which lack Sp1, further confirmed that the activation of promoter II by glucose involves increased binding of Sp1 to the GC-rich sequence (15).

The nuclear extracts from glucose-treated cells exhibit increased Sp1 binding activity (16). This increase in binding activity is not due to glucose-mediated changes in the amount of Sp1 in the nucleus but to an increase in an activity that modifies Sp1 so that it binds more effectively to the promoter sequence (16). This Sp1 modifying activity is inhibited by okadaic acid and phosphatase inhibitor 2 and is 38–42 kDa. The catalytic subunit of type 1 protein phosphatase, 38 kDa, also increased the ability of Sp1 to bind to promoter II. Treatment of nuclear extract with antibodies to the catalytic subunit partially suppressed binding of nuclear protein to the Sp1 sequences. These studies suggest that glucose causes enhanced binding of Sp1 to promoter II and transcriptional activation, both of which are a result of glucose-induced dephosphorylation of Sp1 by type 1 phosphatase.

CONCLUDING REMARKS

Long-chain fatty acids are involved in all aspects of biological function. Specialization of tissues in higher eukaryotes requires complex and tissue-specific responses of fatty acid synthesis at different developmental stages and under different physiological conditions. ACC, as the rate-limiting enzyme for the biogenesis of long-chain fatty acid synthesis, is regulated through a complex array of control mechanisms. These control mechanisms include the following: allosteric regulation of the enzyme by cellular metabolites; regulation by covalent modification; and regulation of both transcription and translation. The ACC-α gene contains two promoters that respond differentially to external agents such as hormones nutrients; these responses lead to the tissue-specific generation of multiple forms of ACC mRNA. The occurrence of five gene products that have the same coding regions but different 5′-end untranslated regions suggests a new set of regulatory mechanisms for the ACC system. The identity of the agents that control differential splicing of the primary ACC-α transcripts in different tissues and the mechanisms by which they exert their effects are unknown. Nevertheless, the occurrence of multiple forms of ACC

mRNA suggests that the tissue-specific regulation of ACC activity and control of the rate of fatty acid synthesis starts at the gene level. In addition to these fundamental questions with regard to ACC-α, we are now faced with a new form of ACC, namely ACC-β. Mechanisms involved in the regulation of ACC-β at the protein and gene level are totally unknown. Even the basic regulatory mechanisms that have already been elucidated with regard to ACC-α remain to be investigated for ACC-β. Regulation of the activities of both forms must be understood before we can begin to understand how the amount of fatty acid in cells is regulated. If ACC-β is indeed a critical factor for mitochondrial fatty acid oxidation, the balance of the activity of these two isoforms should be the factor controlling the amount of fatty acid in the cells. In addition, understanding how the two isoforms are regulated may be a key to understanding how the ACC system controls nonlipogenic cellular events, such as insulin secretion by the β-cells (9) and the energy-sensing mechanisms in the muscle cells (79). This review, therefore, reflects not how much, but how little, we know about the regulatory mechanisms for ACC.

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