Rapid increase of cytosolic content of acetyl-CoA carboxylase isoforms in H9c2 cells by short term treatment with insulin and okadaic acid

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Accepted 30 April 1998

Abbreviations: CPT-1, carnitine palmitoyl transferase-I; ACC, acetyl CoA carboxylase

Abstract

Mammalian acetyl-CoA carboxylase (ACC) is present in two isoforms, α and β , both of which catalyze formation of malonyl-CoA by fixing CO₂ into acetyl-CoA. ACC- α is highly expressed in lipogenic tissues whereas ACC- β is a predominant form in heart and skeletal muscle tissues. Even though the tissue-specific expression pattern of two ACC isoforms suggests that each form may have a distinct function, existence of two isoforms catalyzing the identical reaction in a same cell has been a puzzling question. As a first step to answer this question and to identify the possible role of ACC isoforms in myogenic differentiation, we have investigated in the present study whether the expression and the subcellular distribution of ACC isoforms in H9c2 cardiac myocyte change so that malonyl-CoA produced by each form may modulate fatty acid oxidation. We have observed that the expression levels of both ACC forms were correlated to the extent of myogenic differentiation and that they were present not only in cytoplasm but also in other subcellular compartment. Among the various tested compounds, short term treatment of H9c2 myotubes with insulin or okadaic acid rapidly increased the cytosolic content of both ACC isoforms up to 2 folds without affecting the total cellular ACC content.

Taken together, these observations suggest that both ACC isoforms may play a pivotal role in muscle differentiation and that they may translocate between cytoplasm and other subcellular compartment to achieve its specific goal under the various physio-logical conditions.

Keywords: acetyl-CoA carboxylase, carnitine palmitoyltransferase-I, malonyl-CoA, digitonin, H9c2 cardiac myocyte.

Introduction

Acetyl-CoA Carboxylase (ACC) catalyzes formation of malonyl-CoA from acetyl-CoA and CO₂ which is the rate-limiting step in fatty acid biosynthesis (Wakil *et al.*, 1983; Numa and Tanabe, 1984). Malonyl-CoA serves as a precursor of fatty acid biosynthesis and an intermediate of fatty acid elongation, but it also acts as an allosteric inhibitor of carnitine palmitoyl transferase-I (CPT-I). Located in the outer membrane of mitochondria, CPT-I catalyzes the rate-limiting step in fatty acid uptake and oxidation by mitochondria (Cook, 1984; McGarry *et al.*, 1989). Primary species of mammalian ACC expressed in lipogenic tissues has a molecular weight of 265 kDa (ACC- α) (Lopez-Casillas *et al.*, 1988; Ha *et al.*, 1994b). In contrast, an ACC isoform of 276 kDa (ACC- β) is a predominant form in catabolic tissues such as heart and skeletal muscle in which fatty acid synthesis rate is very low (Thampy, 1989; Bianchi *et al.*, 1990; Saddick *et al.*, 1993; Trumble *et al.*, 1995). Since malonyl-CoA, produced only by ACC, inhibits the activity of CPT-I and since fatty acid oxidation is a major source of energy production in heart and muscle tissues, ACC- β was postulated to control fatty acid oxidation rather than biosynthesis.

ACC- α and β are generally considered cytosolic enzymes, and both carry out an identical reaction, formation of malonyl-CoA (Thampy, 1989). This phenomenon raised an interesting question. In several tissues such as liver, heart, skeletal muscle in which two forms are distinctively detected, first, what is the function of malonyl-CoA generated by each isoform? Second, if there is difference in the role, what kind of regulatory mechanism should exist to specifically recognize or discriminate malonyl-CoA produced by each isoform? The clues for these questions were obtained from recent cloning of ACC- β cDNA from human skeletal muscle tissue (Ha *et al.*, 1996; Widmer *et al.*, 1996). The comparison of amino acid sequences of two isoforms revealed that the first

N-terminal 200 amino acids of ACC- β has no homology at all with the first N-terminal 70 amino acids of ACC- α whereas the remaining region containing all known functional domains shares approximately 70% identity. The molecular difference arises from N-terminal region of ACC. In addition, N-terminus of ACC- β , very rich in amino acids with hydroxyl group likely reflecting the nature as target sites of intensive phosphorylation, contains a membrane-anchoring motif. On the basis of these observation, it was hypothesized that under certain conditions, ACC- β may associate with mitochondria outer membrane in such a way that malonyl-CoA could specifically regulate CPT-1 in a defined area and that this association may be controlled by reversible phospho-rylation of ACC at its N terminus (Ha *et al.*, 1996).

The present study was undertaken to test this hypothesis using rat H9c2 embryonic myocytes. We have observed that these cells expressed only ACC- α in the stage of myoblast, but ACC- β was dramatically induced with a concomitant increase of ACC- α level during differentiation into myotubes. ACC activity increased approximately 7 folds during myogenic differentiation. The expression level of both ACC forms was correlated with the differentiation status of these cells suggesting that not only ACC- β but also ACC- α may play a pivotal role in muscle differentiation. Furthermore, our results revealed that both ACC forms were present not only in cytoplasm but also in other subcellular compartments. In order to test the change of ACC amount in cytoplasm, H9c2 cells treated with various compounds which modulate the phosphorylation state of ACCs were permeabilized with digitonin, and cytosolic protein extracts were analyzed by immunoblot analysis. The result showed that among the various tested compounds, short term treatment with insulin or okadaic acid increased the cytosolic level of both ACC isoforms approximately 2 folds. Under these conditions, total cellular amount of each ACC isoform was not affected. These observations suggest existence of the novel regulatory mechanism controlling subcellular distribution of ACCs. Nature of this mechanism is discussed.

Materials and Methods

Materials

Digitonin, 8-(4-chlorophenylthio)-adenosine 3', 5'-cyclic monophosphate (8-CPT cAMP), 5-amino-4-imidazole-carboxamide ribotide (5' AICAR), okadaic acid and streptavidin-alkaline phosphatase conjugates were purchased from Sigma. Dulbesco's modified Eagle's medium/F-12, horse serum and donor calf serum were purchased from GIBCO/BRL. LY 29400 and insulin were purchased from Calbiochem. $\text{NaH}^{14}\text{CO}_3$ (55 mCi/mmol)

was from ICN.

Cell culture

H9c2 cardiac myoblasts were grown in Dulbesco's modified Eagle's medium/F-12 containing 10% donor calf serum, 100 IU/ml penicillin and 100 $\mu\text{g}/\text{ml}$ streptomycin. After reaching confluence, cells were induced to differentiate to myotubes by changing media to fresh medium containing 1% horse serum. Medium was changed every two days. For the short treatment of hormone or drug, cells were washed with cold phosphate-buffered saline (PBS) and then incubated for the 20 min in Krebs-Ringer buffer (25 mM HEPES, pH 7.4, 5 mM glucose, 118 mM NaCl, 4.8 mM KCl, 1.3 mM CaCl_2 , 1.2 mM KH_2PO_4 , 1.3 mM MgSO_4 , 5 mM NaHCO_3 , and 0.07% BSA) containing various compounds.

Protein extraction

In order to extract cytosolic proteins, H9c2 cells were washed with cold phosphate-buffered saline, and then extraction buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.25% sucrose, 0.4 mg/ml digitonin, and 1.5 mM phenylmethylsulfonyl fluoride) was added to culture dish and incubated on ice for the indicated period of time. After collection of this buffer, the remaining cells were solubilized with extraction buffer containing 1% SDS without digitonin. To extract total cellular proteins, whole cells were directly solubilized with the extraction buffer containing 1% SDS.

Acetyl-CoA Carboxylase assays

Fifty micrograms of protein extracts were incubated in a final volume of 100 μl containing 50 mM sodium phosphate, pH 7.0, 10 mM citrate, 8 mM magnesium sulfate, 1 mM dithiothreitol, 1 mg/ml BSA, 2.25 mM ATP, 0.5 mM acetyl-CoA, and 5 mM ^{14}C -sodium bicarbonate at 37°C for 10 min. A reaction mixture without ATP, acetyl-CoA, and sodium bicarbonate was preincubated for 30 min. at 37°C. The reaction was stopped by adding 50 μl of 6 N HCl, and acid-stable malonyl-CoA was measured by scintillation counter. One unit of acetyl-CoA carboxylase activity was defined as the micromoles of malonyl-CoA formed per minute at 37°C.

Immunoblot analysis

The extracted proteins were separated by 5 % SDS-PAGE and then transferred to nitrocellulose membrane by electrotransfer. Membrane was incubated in TBST buffer (10 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 0.05% Tween-20) containing 1% BSA for 1 h followed by another 1 h incubation with TBST buffer containing streptavidin alkaline phosphatase-conjugate. Membrane was washed three times for 1 h with TBST buffer to remove excess streptavidin alkaline phosphatase-conjugate. Then, alkaline phosphatase reaction was

performed until bands with a distinctive color were observed.

Results

Expression of ACC isoforms during H9c2 cell myogenic differentiation

Since ACC- β is highly expressed in heart and muscle tissues (Thampy, 1989; Bianchi *et al.*, 1990; Trumble *et al.*, 1995), we have used H9c2 cardiomyocytes to study the regulatory mechanisms of ACC isoforms. H9c2 myoblasts were grown in a medium containing 10 % donor calf serum. When confluent, myoblast was incubated in a 1% horse serum containing medium. Under these conditions, myoblasts elongated and fused with each other, and multinucleate myotube formation was observed in 3 days. In 6 days, these cells were fully differentiated. Protein extracts were harvested during differentiation, and immunoblot analysis was performed using streptavidin alkaline phosphatase conjugate because avidin binds to

biotin moiety of all known carboxylases with high affinity. The result showed that only ACC- α was detected in myoblast, and that its expression level increased with a dramatic induction of ACC- β during differentiation (Figure 1). ACC activity also increased approximately 7 folds. We were not able to distinguish to what extent each isoform contributed to the increased activity.

LY294002, the specific inhibitor of phosphatidylinositol-3 kinase which thereby blocks the signal transduced by insulin in many cell types, was

Figure 1. Expression pattern of ACC isoforms and ACC activity during H9c2 myogenic differentiation. H9c2 cells were grown and induced to differentiate as described in the Method section. Cytosolic proteins were extracted using digitonin-containing buffer at day 0 (D0), 3 (D3), and 6 (D6) of differentiation. Immunoblot analysis using streptavidin-alkaline phosphatase and ACC activity assay were performed. The amount of both ACC isoforms increased during differentiation accompanying 7-fold induction of ACC activity. Data shown are mean values $\pm$ S.E. of three experiments.

Figure 2. The effect of LY294002 on differentiation of H9c2 cells. Pictures were taken at the indicated time. A: H9c2 myoblast. B: H9c2 was induced to differentiate for 6 days. C: LY 294002 was added to differentiation medium to give a final concentration 25 nM, and cells were induced to differentiate for 6 days.

Figure 3. Expression pattern of ACC isoforms in H9c2 cell treated with LY294002.. H9c2 cells were induced to differentiate for total 6 days under the indicated conditions. Proteins were extracted using digitonin containing buffer as described in Method section, and 20 μ g of protein extracts were analyzed by immunoblot analysis using streptavidin-alkaline phosphatase. Lane 1-3: H9c2 cells were induced to differentiate for 3 days and then LY294002 was added to medium for another 3 days, Lanes 1, control; 2, LY294002 6.4 nM; 3, LY294002 12 nM. Lanes 4-8: H9c2 cells were induced to differentiate for 6 days in the presence of indicated concentration of LY294002. Lanes 4, control; lane 5, 3.2 nM; lane 6, 6.4 nM; lane 7, 12.5 nM; lane 8, 25 nM. LY294002 treatment indeed suppressed H9c2 cell differentiation in a concentration-dependent manner.

previously reported to block differentiation of L6E9 skeletal muscle cells (Vlahos *et al.*, 1994; Kaliman *et al.*, 1996). This observation prompted us to investigate the effect of LY294002 on ACC isoform expression in H9c2 myotubes during their differentiation. When cells were induced to differentiate in the presence of medium containing 25 nM of LY294002, no myotube formation was observed (Figure 2). Under these condition, the level of ACC isoforms was analyzed. By LY294002 treatment through entire differentiation scheme of 6 days or for last 3 days of differentiation, the expression level of both ACC forms dramatically decreased in a concentration-dependent manner (Figure 3). H9c2 myogenic differentiation was also suppressed by LY294002 in a concentration-dependent manner. These observations indicate that the expression level of both ACC forms was correlated with myogenic differentiation status. Thus, not only ACC- β but also ACC- α may play a pivotal role in muscle differentiation.

Release pattern of ACCs after H9c2 cell permeabilization with digitonin

In order to study the subcellular distribution of ACCs, we decided to measure ACC amount present in cytoplasm under the various conditions. Digitonin has been used to permeabilize cells in order to extract cytosolic proteins (Bijleveld *et al.*, 1987). Since the incubation time of cells with digitonin varies several seconds to minutes depending on researchers (Bijleveld *et al.*, 1987), we first determined the optimal condition for digitonin extraction method by comparing the ACCs amount present in cytosolic fraction and the rest insoluble fraction. Fully differentiated H9c2 cells were incubated with digitonin containing buffer (50 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.25% sucrose, 0.4 mg/ml digitonin, and 1.5 mM phenylmethylsulfonyl fluoride) for various time intervals, 15 sec-15 min, and then, the buffer were collected for further analysis. This portion

Figure 4. ACC extraction condition with digitonin containing buffer

A. H9c2 cells were fully differentiated in 6 well culture plates, and medium was removed. After washing the culture plate with PBS, 100 μ l of digitonin extraction buffer was added to each well, and incubated on ice for the indicated period of time. Then, the buffer was quantitatively recovered and was considered cytosolic proteins. One fifth volume (20 μ l) was subjected for 5 % SDS-PAGE for detection of ACCs by immunoblot analysis. PC; mitochondrial pyruvate carboxylase.

B. The remaining cells after digitonin-extraction were briefly washed with PBS, and solubilized with 100 μ l of buffer containing 1 % SDS. One fifth volume (20 μ l) was subjected for 5 % SDS-PAGE for detection of ACCs by immunoblot analysis. PC; mitochondrial pyruvate carboxylase.

C. The graph shows the relative intensities of ACC isoform bands measured by densitometer at each condition. digitonin: proteins extracted with digitonin containing buffer, SDS: proteins solubilized with SDS containing buffer after cytosolic protein extraction. Data shown are mean values $\pm$ S.E. of three experiments.

was considered cytosolic fraction. Proteins present in the remaining cells after digitonin extraction were extracted by solubilization of the remaining cells with 1% SDS containing buffer. In order to determine the release pattern of ACCs by digitonin, same volume of each fraction was subjected to 5% SDS-PAGE followed by

Figure 5. Changes in the level of cytosolic ACC isoforms by 8CPT-cAMP, 5' AICAR, insulin and okadaic acid. H9c2 cells at day 6 were treated with different concentration of indicated compounds for 20 min. Twenty micrograms of cytosolic proteins were analyzed for detection of ACCs amount by immunoblot analysis. In order to extract total cellular proteins, cells were directly solubilized with SDS containing buffer. The relative band intensities of both ACC forms observed at the highest concentration condition of each additive were measured by densitometer, and expressed as percentage of those at untreated conditions. Data shown are mean values $\pm$ S.E. of three experiments.

immunoblot analysis (Figure 4). The result showed that the amount of ACCs in cytosolic fraction increased up to 5 min incubation, and that of ACCs in SDS fraction decreased until same incubation time. This observation indicates that it is necessary to incubate H9c2 cell with digitonin buffer at least for 5 min in order to completely remove cytosolic proteins. Even after 5 min incubation, both ACC forms were also detected in insoluble fraction indicating that ACCs were associated with certain sub-cellular compartment(s) other than cytoplasm. Under the longer incubation than 5 min, mitochondrial pyruvate carboxylase was detected indicating that mitochondria was also permeabilized. Therefore, for further analysis, we have used 5 min incubation time.

Rapid changes of cytosolic concentration of ACC isoforms under short term treatment of insulin and okadaic acid

Since ACC- α and β both catalyze formation of malonyl-CoA, and their expression pattern is tissue-specific (Ha *et al.*, 1996), it is reasonable to predict that malonyl-CoA produced by each ACC isoform participates in

different cellular activities. As an attempt to reveal the regulatory mechanisms which may provide specific roles for each ACC isoform, we have tested the hypothesis that ACC- β associates with the outer membrane of mitochondria where its product, malonyl-CoA, specifically regulates the closely located CPT-1 and that this association is controlled by the phosphorylation state of ACC- β N terminus (Ha *et al.*, 1996). In order to test whether translocation of ACC isoforms from cytoplasm to other subcellular compartments actually occurs, we have measured the amount of ACC isoforms present in cytoplasm under the short term treatment of H9c2 cells with various compounds that are known to change phosphorylation state of ACCs including cAMP analogue (8-CPT cAMP), 5-amino-4-imidazole-carboxamide ribotide (5' AICAR), an 5' AMP analogue and activator of 5'-AMP protein kinase (Witters *et al.*, 1992; Witters *et al.*, 1988), insulin, and okadaic acid (Figure 5). cAMP-dependent protein kinase and 5'-AMP-dependent protein kinase are two major kinases for ACC phosphorylation (Ha *et al.*, 1994; Kim *et al.*, 1989). Insulin is generally believed to stimulate the dephosphorylation of

ACC by inactivation of 5' AMP protein kinase (Witters *et al.*, 1988; Witters *et al.*, 1992). Among the tested compounds, insulin and okadaic acid treatment of H9c2 myotube for 20 min increased the level of both ACC isoforms present in cytoplasm approximately 2 fold in a concentration-dependent manner without affecting total cellular level of ACC isoforms. Other reagents such as cAMP, or 5' AICAR had no effect. These results appeared to be contradictory since insulin have an opposite effect compared to okadaic acid which stimulate phosphorylation state by inhibiting protein phosphatase 1 and 2A. Furthermore, 5' AICAR incubation together with insulin did not overcome insulin effect whereas LY 294002, which blocks insulin signal transduction by inhibiting phosphatidylinositol-3 kinase, completely abolished insulin effect. This particular result suggest that the observed insulin effect is not due to dephosphorylation of ACC. Taken together, these observations suggest that ACC- α and β may translocate between cytoplasm and other subcellular compartment by another unknown mechanism rather than by reversible phosphorylation, and possible mechanisms for these activities are discussed.

Discussion

The regulatory mechanisms of ACC- α are extremely complex and sophisticated. Its gene has two promoters, promoter I with inducible nature under lipogenic conditions and promoter II which constitutively expresses (Luo *et al.*, 1989). From these two promoters, at least five distinct messages with different 5' untranslated region are produced (Lopez-Casillas and Kim, 1989). In addition, ACC- α is regulated by covalent modification of phosphorylation/dephosphorylation (Kim *et al.*, 1989; Ha, 1994b), and subject to allosteric regulation by cellular metabolites including citrate and acyl-CoA (Carson and Kim, 1979; Beaty and Lane, 1983). As described here, ACC- α alone has a wide spectrum of gene expression level and enzyme activity to fulfill cellular malonyl-CoA requirements under the various physiological conditions. Nevertheless, existence of isoform catalyzing the identical reaction gives a rise to the puzzling role of ACCs. Recent cloning of ACC- β cDNA revealed that it has a gene distinct from ACC- α gene (Ha *et al.*, 1996; Widmer *et al.*, 1996); human ACC- β gene is located on the chromosome 12 (Widmer *et al.*, 1996) whereas ACC- α gene is present on chromosome 17 (Milatovich *et al.*, 1988). As a regulatory mechanism explaining how cell can distinguish malonyl-CoA generated by each isoform when both isoforms are expressed in a same cell, Kim and coworkers hypothesized the compartmentalization of malonyl-CoA produced by ACC- β (Ha *et al.*, 1996). However, in this study, we presented several observations to suggest that not only ACC- β but also ACC- α could be regulated by a similar translocation mechanism.

Our results suggest that phosphorylation/dephosphorylation of ACC cannot merely explain or be responsible for our observations because change of the cytosolic amount of ACC isoforms were not consistent with phosphorylation state of ACCs. cAMP and 5' AICAR which activates two major protein kinases for ACC had no effect whereas okadaic acid, the inhibitor of phosphatase, showed the significant effect. Furthermore, insulin which stimulate dephosphorylation of ACC by inhibiting 5'-AMP dependent protein kinase also increased the cytosolic content of ACC. Therefore, it is very possible that another mechanism may be involved in this translocation activity. Insulin has pleiotropic effects and phosphatidylinositol 3-kinase plays a key role in insulin signal transduction. Accumulating evidences indicate that this kinase is involved in the rearrangement of cytoskeleton by direct interaction with tubulin or α -actinin (Kapeller *et al.*, 1995; Fukami *et al.*, 1996). In addition, other evidences show that this kinase act as a regulator of endocytosis (Li *et al.*, 1995). We have observed that LY294002, not 5' AICAR, completely abolished the translocation activities induced by insulin. Furthermore, okadaic acid not only act as protein phosphatase inhibitor, but also it has been shown to disrupt the cytoskeleton of hepatocytes (Holen *et al.*, 1993). These observations suggest that rather than phosphorylation and dephosphorylation, cytoskeleton rearrangement could be responsible for the observed ACC translocation activities. Therefore, in addition to covalent modification by reversible phosphorylation, allosteric control and differential gene expression, direct protein-protein interaction involving cytoskeleton may regulate ACC. The changes in cytosolic ACC- α and β appear to occur in a coordinate manner (Figure 5) although we cannot rule out the possibility that either α or β alone could be translocated under other conditions. A recent report shows that ACC- α and β form a heterodimer in hepatocytes (Iverson *et al.*, 1990). It is thus possible that ACC may form homodimer of α or β as well as heterodimer depending on the physiological conditions. The relationship between ACC translocation activity and these dimer formation, the translocation target site, and the enzyme activities in other subcellular location have yet to be determined in order to fully understand the function of two isoforms.

Identification of isoform further increased the complexity of understanding the regulatory mechanisms as well as the role of ACCs especially when both forms are expressed in a same cell. ACC- α and β catalyzes the same reaction and their overall homology is approximately 70% (Ha *et al.*, 1996). In addition, mRNA size of both ACCs is about 10 kilobase long (Ha *et al.*, 1994b; Ha *et al.*, 1996). Such similarity in biochemical reaction and protein/gene sequences has made it extremely difficult to detect the existence of an isoform. As a consequence, every experiment regarding ACC up to

now has been performed without consideration of ACC- β . Therefore, the more careful examination of each data is required to reveal the precise control mechanisms of ACC.

Acknowledgement

This work was supported partially by a grant from the Basic Medical Research Promotion fund of Korean Ministry of Education (L21), a grant from Korea Science and Engineering Foundation (96-0403-14-01-3).

References

- Beatty, N. B. and Lane, M. D. (1983) Kinetics of activation of acetyl-CoA carboxylase by citrate. Relationship to the rate of polymerization of the enzyme. *J. Biol. Chem.* 268: 13043-13050
- Bianchi, A., Evans, J. L., Iverson, A. J., Nordlund, A. G., Watts, T. D. and Witters, L. A. (1990) Identification of an isozymic form of acetyl-CoA carboxylase. *J. Biol. Chem.* 265: 1502-1509
- Bijleveld, C. and Geelen, M. J. (1987) Measurement of acetyl-CoA carboxylase activity in isolated hepatocytes. *Biochim. Biophys. Acta* 918: 274-283
- Carson, C. A. and Kim, K.-H. (1979) Differential effects of metabolites on the active and inactive forms of hepatic acetyl-CoA carboxylase. *Arch. Biochem. Biophys.* 164: 490-501
- Cook, G. A. (1984) Differences in the sensitivity of carnitine palmitoyltransferase to inhibition by malonyl-CoA are due to differences in K_i values. *J. Biol. Chem.* 259: 12030-12033
- Fukami K., Sawada N., Endo T. and Takenawa T. (1996) Identification of a phosphatidylinositol 4, 5-bisphosphate-binding site in chicken skeletal muscle α -actinin. *J. Biol. Chem.* 271: 2646-2650
- Ha, J., Daniel, S., Broyles, S. S. and Kim, K.-H. (1994a) Critical phosphorylation sites for acetyl-CoA carboxylase activity. *J. Biol. Chem.* 269: 22162-22168
- Ha, J., Daniel, S., Kong, I.-S., Park, C.-K., Tae, H.-J. and Kim, K.-H. (1994b) Cloning of human acetyl-CoA carboxylase cDNA. *Eur. J. Biochem.* 219: 297-306
- Ha, J., Lee, J.-K., Kim, K.-S., Witters, L. A. and Kim, K.-H. (1996) Cloning of human acetyl-CoA carboxylase β and its unique function. *Proc. Natl. Acad. Sci. USA* 93: 11466-11470
- Holen, I., Gordon, P. B. and Seglen, P. O. (1993) Inhibition of hepatic autophagy by okadaic acid and other protein phosphatase inhibitors. *Eur. J. Biochem.* 215: 113-122
- Kaliman, P., Vinals, F., Testar, X., Palacin, M. and Zorzano, A. (1996) Phosphatidylinositol 3-kinase inhibitors block differentiation of skeletal muscle cells. *J. Biol. Chem.* 271: 19146-19151
- Kapeller R., Toker A., Cantley L. C. and Carpenter C. L. (1995) Phospho-inositide 3-kinase binds constitutively to α/β -tubulin and binds to γ -tubulin in response to insulin. *J. Biol. Chem.* 270, 25985-25991
- Kim, K.-H., Lopez-Casillas, F., Bai, D.-H., Luo, X. and Pape, M. E. (1989) Role of reversible phosphorylation of acetyl-CoA carboxylase in long chain fatty acid synthesis. *FASEB J.* 3: 2250-2256
- Li, G., D'souza-schorey, C., Babieri, M. A., Roberts, R. L., Klippel, A., Williams, L. T. and Stahl, P. D. (1995) Evidence for phosphatidylinositol 3-kinase as a regulator of endocytosis via activation of Rab5. *Proc. Natl. Acad. Sci. USA* 92: 10207-10211
- Lopes-Casillas, F., Bai, D.-H., Luo, X., Kong, I.-S., Hermodson, M. A., and Kim, K.-H. (1988) Structure of the coding sequences and primary amino acid sequences of acetyl-coenzyme A carboxylase. *Proc. Natl. Acad. Sci. USA* 85: 5784-5788
- Lopez-Casillas, F. and Kim, K.-H. (1989) Heterogeneity at the 5' end of rat acetyl-coenzyme A carboxylase mRNA. Lipogenic conditions enhance synthesis of a unique mRNA in liver. *J. Biol. Chem.* 264: 7176-7184
- Luo, X., Park, K., Lopez-Casillas, F. and Kim, K.-H. (1989) Structural features of the acetyl-CoA carboxylase gene: mechanisms for generation of mRNA with 5' end heterogeneity. *Proc. Natl. Acad. Sci. USA* 86: 4042-4046
- McGarry, J. D., Woeltje, K. F., Kuwajima, M. and Foster, D. W. (1989) Regulation of ketogenesis and renaissance of carnitine palmitoyltransferase. *Diabetes Metab. Rev.* 5: 271-284
- Milatovich, A., Platter, R., Heerema, N. A., Palmer, C. G., Lopez-Casillas, F. and Kim, K.-H. (1988) Localization of the gene for acetyl-CoA carboxylase to human chromosome 17. *Cytogenet. Cell Genet.* 48: 190-192
- Numa, S. and Tanabe, T. (1984) In *Fatty Acid Metabolism and Its Regulation* (Numa, S. ed.), pp. 1-27, Elsevier Science Publishing Co., Inc., New York
- Saddick, M., Gamble, J., Witters, L. and Lopaschuck, G. D. (1993) Acetyl-CoA carboxylase regulation of fatty acid oxidation in the heart. *J. Biol. Chem.* 268: 25836-25846
- Thampy, K. G. (1989) Formation of malonyl coenzyme A in rat heart. Identification and purification of an isozymic of acetyl-CoA carboxylase from rat heart. *J. Biol. Chem.* 264: 17631-17634
- Trumble, G. E., Smith, M. A. and Winder, W. W. (1995) Purification and characterization of rat skeletal muscle acetyl-CoA carboxylase. *Eur. J. Biochem.* 231: 192-198
- Vlahos, C. J., Matter, W. F., Hui, K. Y. and Brown, R. F. (1994) A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4-morpholinyl)-1-phenyl-4h-1-benzopyran-4-one (LY 294002). *J. Biol. Chem.* 269: 5241-5248
- Wakil, S. J., Stoops, J. K. and Joshi, V. C. (1983) Fatty acid synthesis and its regulation. *Annu. Rev. Biochem.* 52: 537-579
- Widmer, J., Fassihi, K. S., Schlichter, S. C., Wheeler, K. S., Crute, B. E., King, N., Natlie-McMenemy, N., Noll, W. W., Daniel, S., Ha, J., Kim, K.-H. and Witters, L. A. (1996) Identification of a second acetyl-CoA carboxylase gene. *Biochem. J.* 316: 915-922
- Witters L. A. and Kemp B. E. (1992) Insulin activation of acetyl-CoA carboxylase accompanied by inhibition of the 5' AMP-activated protein kinase. *J. Biol. Chem.* 267: 2864-2867
- Witters L. A., Watts T. D., Daniels D. L. and Evans J. L. (1988) Insulin stimulates the dephosphorylation and activation of acetyl-CoA carboxylase. *Proc. Natl. Acad. Sci. USA* 85: 5473-5477