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*Originals*

# Hepatic expression of CCAAT/enhancer binding protein $\alpha$: hormonal and metabolic regulation in rats

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**Summary** There is a significant body of evidence which suggests that the α-isoform of the CCAAT/enhancer binding protein (C/EBPα) plays a central regulatory role in energy metabolism in the liver. However, there is little information available regarding regulation of its expression in this tissue. In this study, we examined the effect of hormones and diabetes on its expression in rat H4IIE hepatoma cells and in rat liver. Treatment of H4IIE cells with dexamethasone led to a threefold increase in C/EBPα mRNA within 4 h. Insulin treatment produced a bi-phasic response, initially reducing mRNA levels up to the 4 h time point, but after 8 h a twofold increase in C/EBPα mRNA was observed. Treatment with 8-chlorophenylthio-cAMP produced a twofold induction of C/EBPα mRNA after 8 h. Western analysis indicated that the changes in mRNA in response to hormonal treatment generally resulted in corresponding alterations in C/EBPα protein levels. Finally, we observed an inhibition of C/EBPα gene expression in streptozotocin-diabetic rat liver, reflected by a decrease in both mRNA and protein levels that were partially reversed by insulin treatment. These results indicate that the expression of C/EBPα in liver is under complex control by both hormonal and metabolic signals, which is consistent with its role as a *trans*-regulator of genes which play a role in energy metabolism. [Diabetologia (1997) 40: 1117–1124]

**Keywords** CCAAT/enhancer binding protein, gene expression, transcription factor, diabetes, liver.

The α-isoform of the CCAAT/enhancer binding protein (C/EBP) is a member of the basic region-leucine zipper family of transcription factors. Initially it was identified as a liver-enriched protein, but further analysis indicated that it was also highly expressed in adipose and placental tissue, and at lower levels in small intestine and lung [1]. With the exception of lung, all of these tissues play a major role in different aspects of energy balance. Liver and adipose tissue are major sites for the storage and release of metabolic fuels, the small intestine is involved in fuel uptake from the diet, and the placenta plays a critical role in fetal nutrition. The expression in lung can also be explained in this context, since it is actively involved in the synthesis of surfactant lipids. It is of further interest that C/EBPα binds to and transactivates several metabolically important gene promoters, including phosphoenolpyruvate carboxykinase [3], serum albumin [4], the insulin responsive GLUT2, GLUT4 [5], alcohol dehydrogenase [6], several adipocyte-specific genes [7], and C/EBPα itself [8].

While several C/EBP isoforms co-exist in liver, with the two most abundant being the α- and β-isoforms [9], data from knockout mice indicate that C/EBPα plays a significantly more important role in the regulation of metabolism than the β-isoform. Mice homozygous for the targeted deletion in the C/EBPα gene have no detectable amounts of liver glycogen,

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*Abbreviations:* C/EBP, CCAAT/enhancer binding protein; STZ, streptozotocin; PBS, phosphate-buffered saline; CPT-cAMP, 8-(4-chlorophenylthio) cyclic adenosine monophosphate; CREB, cAMP response element binding protein; PEPCK, phosphoenolpyruvate carboxykinase.

reduced expression of hepatic glycogen synthase and brown adipose uncoupling protein genes, and developmentally delayed expression of hepatic phosphoenolpyruvate carboxykinase and glucose 6-phosphatase genes [10]. The mice die within 8 h of birth from hypoglycaemia. Conversely, C/EBPβ knockout mice are healthy and show no gross histological abnormalities, the only detectable abnormality being a susceptibility to infection [11]. Thus, most of the data collected to date concerning the biological properties of this transcription factor support the hypothesis developed by McKnight and colleagues [2] that C/EBPα may be a central regulator of energy metabolism.

One would predict that, since the expression level of the target genes for this transcription factor are for the most part under tight control, including hormonal regulation, the expression level of C/EBPα would also be regulated. While some studies have examined the hormonal regulation of its expression in adipocytes and related cell lines [12, 13], very little is known concerning its regulation in liver. In this study, we examined the hormonal regulation of hepatic C/EBPα in the intact rat and in rat hepatoma cells. Additionally, we also examined the expression of this transcription factor in streptozotocin (STZ)-diabetic rat liver, used as a model in which to examine the sensitivity of the gene's expression to an altered metabolic state.

## Materials and methods

*Cell culture.* Rat hepatoma H4IIE cells were cultured in DMEM/F12 medium (Gibco-BRL, Burlington, Ontario, Canada) supplemented with 5 % fetal calf serum and 5 % calf serum. When the cells reached 80 % confluency, they were switched to serum-free DMEM/F12 media, and hormonal treatments were initiated 24 h later. Following hormone treatment, the cells were washed twice in phosphate buffered saline (PBS), scraped off the plates in 1 ml of PBS, and then centrifuged at $12\,000 \times g$ for 30 s. Total cellular RNA was prepared using the TriZol (Gibco-BRL) reagent according to manufacturer's instructions, and total cellular protein lysates for Western analysis were prepared as described previously [14].

*Treatment of animals.* Principles of laboratory animal care were followed, and the protocols were approved by the University of Saskatchewan Committee on Animal Care and Supply. Male Sprague-Dawley rats (150–200 g) were purchased from Charles River Laboratories (St. Constant, Quebec, Canada) and randomly placed into control and treatment groups. Rats were injected with theophylline (30 mg per kg body weight) in a sterile PBS vehicle, followed by injection with 8-(4-chlorophenylthio) cyclic adenosine monophosphate (8 CPT-cAMP, Sigma, Mississauga, Ontario, Canada), also in PBS vehicle at a dose of 30 mg/kg body weight, at 30-min intervals over a 90-min period. Control rats received a PBS injection at the same time intervals. Following treatment, the rats were killed and part of the liver was quickly frozen in liquid nitrogen and stored at − 80 °C until used for isolation of RNA, while the remainder was processed immediately for the preparation of nuclear lysates for Western analysis (see below).

Rats were made diabetic by a single intraperitoneal injection of streptozotocin (Sigma) (80 mg/kg body weight) in 100 mmol/l sodium citrate pH 4.0, 150 mmol/l NaCl vehicle. The control group was injected with the same volume of buffer only. All rats were tested after 5 days for glucosuria using 5L Chemstrips (Boehringer Mannheim, Laval, Quebec, Canada). All rats in the treatment group tested positive for glucosuria and showed significant weight loss ( > 20 g). Half of the diabetic rats were started on a twice daily regimen of 5 IU of NPH insulin (Connaught Laboratories, Willowdale, Ontario, Canada) injected subcutaneously for 5 days, and monitored daily with Chemstrips to ensure appropriate insulin replacement. The untreated diabetic rats were killed 5–6 days after testing positive for glucosuria. Blood glucose assessment at the time of killing indicated levels over 25 mmol/l in all untreated diabetic rats. The livers obtained from these rats were handled as described in the previous section.

*RNA extraction and analysis.* Total RNA was isolated from rat liver with TriZol reagent (Gibco-BRL) according to the manufacturer's instructions. RNA samples were quantified via optical density readings obtained at 260 nm, and RNA integrity was subsequently checked by agarose/formaldehyde gel electrophoresis to visualize intact 28S and 18S ribosomal RNAs. Further vertification that an equivalent amount of RNA was present in each load was accomplished by RNA slot blot analysis using a radiolabelled 18S rRNA probe [15]. The radioactive signals, which were quantified by counting the corresponding radioactive spot on the membrane in a scintillation counter, were all within 10 % of each other, indicative of proper RNA concentration quantification. Synthesis of antisense RNA probes for C/EBPβ, CREB and PEPCK mRNA and the RNase protection assay used have been described previously [16]. The RNA probe for C/EBPα mRNA was synthesized by ligating a BamHI/PstI fragment of the rat C/EBPα cDNA, containing nucleotides + 131 to + 782 [17], into the same sites in pTZ19R (United States Biochemical Cleveland, Ohio, USA) in an antisense orientation. The resulting vector was linearized with AvaI, which cuts at position + 450 in the C/EBPα cDNA, allowing synthesis of a 370 nt antisense probe which protected a 332 nt region of the C/EBPα mRNA, which is unique to this isoform. Following autoradiography,

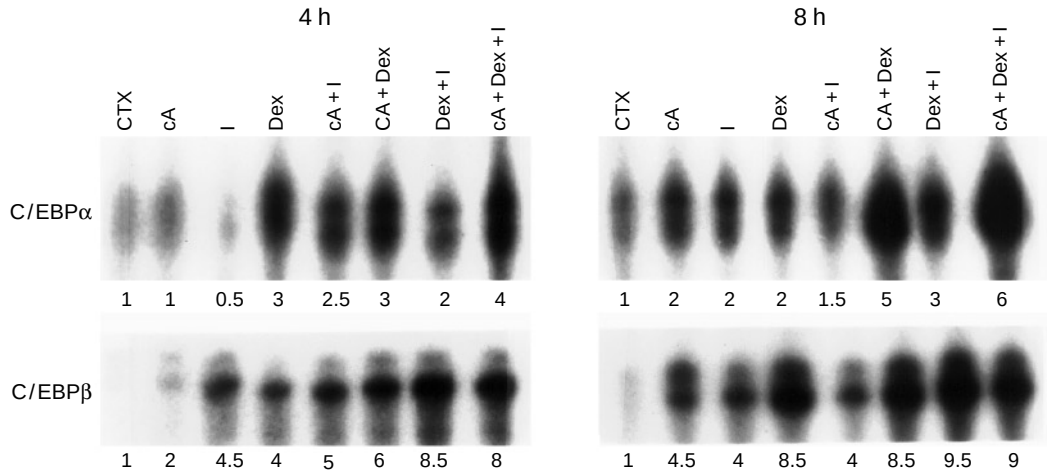


**Fig. 1.** Relative mRNA levels of C/EBP isoforms in hormonally treated H4IIE cells. Cells were treated with either 200 μmol/l CPT-cAMP, 1 nmol/l insulin or 1 μmol/l dexamethasone for either 4 h (left side) or 8 h (right side). Total RNA isolated from the cells was analysed for C/EBPα, C/EBPβ, and PEPCK mRNA levels by ribonuclease protection assay, as indicated in the Methods section. Each panel shows a representative autoradiogram, and the values below the autoradiograms indicate the mRNA level (relative to controls which were assigned a value of 1) averaged from four independent experiments. Ctx, Control; cA, 8-CPT-cAMP; I, insulin; Dex, dexamethasone

results were quantified using a laser densitometer (LKB 2202 Ultrascan, Bromma, Sweden).

*Western analysis.* The preparation of nuclear lysates from fresh rat liver and total cellular protein lysates from H4IIE cells has been described previously [14]. For Western analysis, equivalent amounts of protein were resolved by SDS-PAGE. Resolved protein bands were transblotted onto polyvinyldifluoridene (PVDF) membranes (Polyscreen – DuPont NEN, Mississauga, Ontario, Canada). The PVDF membranes were blocked with 5% reagent-grade fat-free skim milk powder (BioRad, Mississauga, Ontario, Canada) and PBS containing 0.02% Tween-20. Primary and secondary antibody incubations and subsequent washes were carried out using this buffer. Primary anti-sera to C/EBPα, C/EBPβ, CREB and TFIIEα were obtained from Santa Cruz Biotechnology (Santa Cruz, Calif., USA). Goat anti-rabbit IgG horseradish peroxidase conjugate (BioRad) was used as the secondary antibody. Bands were detected by chemiluminescence (DuPont-NEN) and autoradiograms were quantified using an LKB 2202 laser densitometer.

## Results

In most of the experiments described below, we have simultaneously assessed the effects of hormonal and metabolic treatments on both the α- and β-isoforms of C/EBP, since they represent the two most abundant forms in liver and offer an opportunity for comparison of their regulation.

*Effect of hormones on the expression of C/EBP isoforms in rat hepatoma cells.* Rat hepatoma H4IIE cells were used in this study since they respond appropriately to various hormonal stimuli and display many characteristics of differentiated liver cells. These cells were treated for 4 and 8 h with hormones individually and in various combinations, and then analysed for alterations in the level of C/EBPα and C/EBPβ mRNA by RNase protection. Figure 1 shows a representative autoradiogram as well as the averages of four experiments. In the experiments where cells were incubated with hormones for 4 h, dexamethasone was observed to have the most dominant effect on C/EBPα expression in the 4 h incubation study, with insulin treatment resulting in an inhibition of C/EBPα gene expression (Fig. 1). Unexpectedly, cells incubated with insulin and CPT-cAMP combination resulted in an approximately threefold induction, although neither hormone alone had any stimulatory effect. Insulin did inhibit the stimulatory effect of dexamethasone. In the 8 h incubation study, all of the hormones elicited a twofold induction in C/EBPα mRNA levels, and additive effects with cAMP + dex and cAMP + dex + insulin combinations were observed. To summarize, hormone treatments which involved dexamethasone either alone or in combination produced the greatest induction of mRNA levels.

Expression of the C/EBPβ gene was more sensitive to hormonal regulation than C/EBPα at the time points chosen. In the 4 h treatment study (Fig. 1), C/EBPβ mRNA levels were induced twofold by CPT-cAMP, and induced 4- to 5-fold by dexamethasone or insulin. Combination hormone treatment resulted in approximately additive effects. With 8 h treatments, a larger fold induction was observed with CPT-cAMP, and dexamethasone had strong inductive

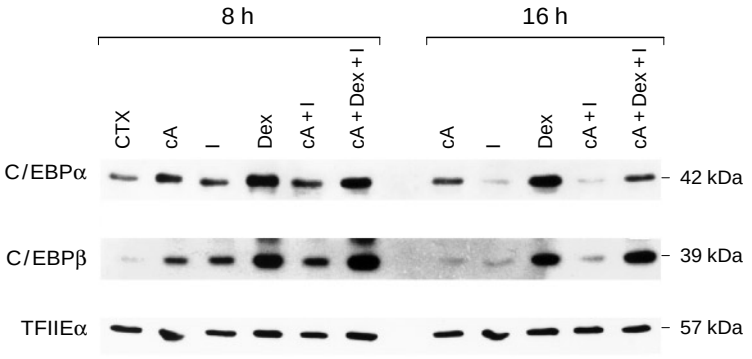


**Fig. 2.** Western analysis of C/EBP isoforms in hormonally treated H4IIE cells. Cells were treated with either 1 mmol/l CPT-cAMP, 1 nmol/l insulin, 1 μmol/l dexamethasone, or combinations thereof for either 8 or 16 h as indicated. Whole cells lysates were prepared and subjected to Western blot analysis, as described in the Methods section. Qualitatively similar results were obtained from four independent experiments. Ctx, Control; cA, 8-CPT-cAMP; I, insulin; Dex, dexamethasone

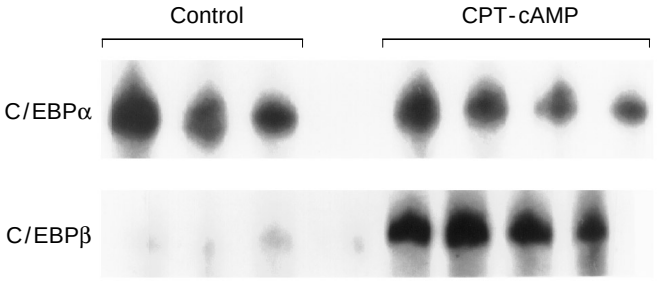


**Fig. 3.** Effect of cAMP on the mRNA levels of C/EBP α and β isoforms in rat liver. Rats were given an intraperitoneal injection of CPT-cAMP/theophylline (CPT-cAMP, $n = 4$) or saline carrier (controls, $n = 3$), and the RNA isolated from the liver was analysed for levels of C/EBPα and β mRNA by RNase protection. Equilibration of RNA between samples was verified by slot blot analysis using an 18S rRNA probe as described in the Methods section

effects alone or in combination with other hormones. Thus, glucocorticoids appear to be the dominant activator of both C/EBPα and β gene expression.

As a control for these hormone treatment experiments, we assessed the changes in PEPCK mRNA, for which the response to hormones has been well-documented. The PEPCK gene was induced by CPT-cAMP and dexamethasone, and insulin inhibited both basal expression and that induced by cAMP or dexamethasone (data not shown). We also assessed CREB mRNA levels in the various RNA samples collected, since we had previously demonstrated that CREB mRNA levels are not hormonally regulated in liver or liver-derived cells [16]. In all treated samples CREB mRNA levels were similar to control levels, providing support for the specificity in the changes in C/EBPα and β mRNA in response to various hormone treatments (data not shown).

Next, we performed Western analysis on nuclear extracts prepared from hormone-treated (8 and 16 h) H4IIE cells to determine whether alterations observed at the level of C/EBP mRNA resulted in corresponding changes in steady-state levels of protein. Similar to the effect on mRNA levels, dexamethasone had the strongest inductive effect on C/EBPα protein levels (4- to 5-fold), while CPT-cAMP had no detectable effect and insulin produced a moderate stimulation after 8 h treatment but was lower than controls after 16 h (Fig. 2). The large positive effect of dexamethasone indicated that the cells retained their protein synthesis capacity despite being placed in serum-free media during the hormone treatments. The changes in C/EBPβ almost identically mirrored those observed for C/EBPα, with the exception being that the dexamethasone effect was slightly delayed (Fig. 2). These changes observed in the two isoforms of C/EBP were shown to be specific, since the levels of the general transcription factor TFIIEα in the same extracts did not fluctuate (Fig. 2).

It should be noted that longer treatment times were chosen to examine the effects of hormones on protein levels, compared with those used to examine the effect on mRNA levels. This was based upon the well-known time delay between changes in gene transcription and changes in protein expression.

*Effect of cAMP bolus on C/EBP isoform mRNA levels in rat liver.* The effects of cAMP on the expression of the two isoforms in H4IIE cells suggested that the β-isoform was more responsive to this second messenger. We decided to examine this further using an in vivo setting. The mRNA levels of C/EBPα and β in livers of rats given a 90-min regimen of CPT-cAMP injections were measured by RNase protection. No significant difference in C/EBPα mRNA levels were detectable between cAMP-treated and control rats (Fig. 3). This correlates with the insignificant effect of cAMP treatment on C/EBPα mRNA observed in H4IIE cells in the 4 h treatment (Fig. 1). The levels of C/EBPβ mRNA were elevated by approximately 10-fold over controls in these same livers (Fig. 3). In this same experiment, the levels of PEPCK mRNA were considerably elevated while CREB

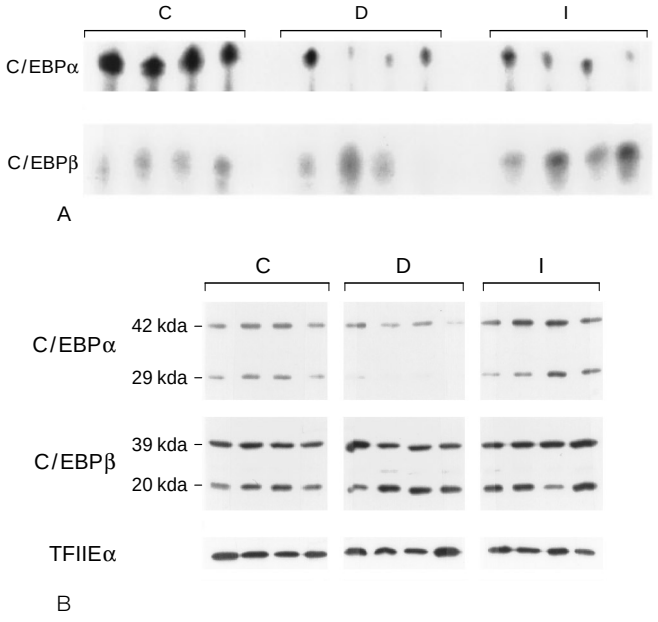


**Fig. 4.** Effect of diabetes and insulin treatment on the levels of C/EBPα and β mRNA and protein levels in rat liver. Rats were made diabetic and subsequently treated with insulin as described in the Methods. (**A**) Total RNA isolated from rat liver and analysed for levels of C/EBPα and β mRNA by RNase protection assay. Prior to analysis, equilibration of RNA was verified as described in the legend to Figure 3. (**B**) Western analysis of C/EBPα, C/EBPβ, and TFIIEα was performed on liver nuclear lysates prepared from the three groups of rats, four rats in each experimental group; C, saline controls; D, STZ-diabetic rats; I, insulin-treated diabetic rats. It should be noted that the nuclear lysates from all three experimental groups were analysed on the same blot, but were "cut and pasted" solely for clarity of presentation. The blots were quantified by densitometry, and the data described in the text

mRNA levels were unchanged (data not shown), both consistent with previous observations [16].

*Effect of streptozotocin-diabetes on C/EBP isoform expression in rat liver.* Since there is considerable evidence to indicate that C/EBPα may be an important regulator of energy metabolism, we examined the hepatic expression of both C/EBP isoforms in streptozotocin-induced diabetes, where significant alterations in nutrient metabolism are present. The relative mRNA levels of C/EBPα and β in the livers from control, diabetic, and insulin-treated diabetic rats are shown in Figure 4 A. The levels of C/EBPβ mRNA were not significantly affected by STZ-diabetes or by insulin treatment. However, in all STZ-diabetic rats tested, the levels of C/EBPα mRNA were decreased 60–70 % relative to controls, as determined by densitometry (values not shown), and insulin treatment only partially reversed this decrease. Analysis of C/EBP protein levels in nuclear extracts prepared from these three groups of rats (Fig. 4 B) were performed to confirm the physiological significance of this decrease. As expected, there was no significant change in the levels of the general transcription factor TFIIEα. It should be noted that two different translated products (39 and 20 kDa) from the C/EBPβ mRNA result from use of alternate AUG initiation codons [18, 19]; however, no change in either species was observed in diabetic or insulin-treated rat livers. However, the levels of C/EBPα in diabetic rat liver nuclei were decreased relative to controls. Similar to C/EBPβ, two major translation products are observed in rat liver. Interestingly, there was a larger ( ~ 50–60 %) decrease in the 29 kDa from compared with a more moderate approximate 25 % decrease in the 42 kDa form of C/EBPα.

## Discussion

Changes in the expression of genes which encode for enzymes, brought about as a result of adaptive responses to changes in nutritional, developmental, or other environmental conditions, are well described. One area in which our basic understanding of these processes is relatively weak is in alterations in transcription factor expression, which are the proteins that transduce the various environmental signals onto the genes. In this study, we examined the regulation of C/EBPα expression, since there is abundant evidence to suggest that this transcription factor plays a critical role in the regulation of energy and nutrient metabolism. While several studies have examined the role of individual hormones on the expression of this gene, no studies have examined the effect of hormone combinations, and most studies have focused on the regulation of expression in adipose-derived cells, where C/EBPα plays a critical role in differentiation.

Our experiments examining the hormonal regulation of C/EBPα expression in rat hepatoma cells indicated that glucocorticoids are the dominant hormone regulator, although this hormone does display interactions with cAMP and insulin. The effect of glucocorticoids on C/EBPα expression appears to be cell-specific. Dexamethasone has been shown to rapidly but transiently reduce the levels of C/EBPα in 3T3-L1 adipocytes and in vivo in white adipose tissue [12], whereas it has no effect on expression of this gene in the IEC-6 rat intestinal epithelium crypt cell line [20]. This opposite effect of glucocorticoids on gene expression in liver and adipose has also been observed in the regulation of PEPCK gene expression [21, 22]. Glucocorticoids are known to inhibit cell growth and division in both regenerating liver and in some liver-derived cell lines [23], and C/EBPα is known to be present at relatively high levels in quiescent, non-dividing rat liver cells [24]. Correlating with our own findings, it has recently been shown that dexamethasone-induced expression of C/EBPα is required for glucocorticoid-mediated cell cycle arrest of rat hepatoma cells [25]. Thus the regulation of

C/EBPα expression by dexamethasone appears to be intimately associated with hepatocyte quiescence.

The role of cAMP and insulin in the regulation of C/EBPα expression in liver has not been previously examined in detail. In the present study, cAMP was observed to have only a marginal effect on expression in hepatoma cells (Fig. 1) and no effect in vivo (Fig. 3), although it did have a significant effect when in combination with dexamethasone (Fig. 1, right panel). Insulin had different effects on C/EBPα expression, depending on the absence or presence of other hormones. By itself, it had slightly inhibitory or negligible effects, it inhibited the dexamethasone effects in the 4 h incubation and slight inhibitory effect on the cAMP-mediated induction in the 8 h study (Fig. 1); in general, however, its effects were small. This is consistent with previous observations made in a mouse model, where feeding a high carbohydrate diet, which led to increased insulin levels, had no effect on C/EBPα expression [26]. In 3T3-L1 adipocytes, insulin produces a rapid reduction in expression of C/EBPα [13].

It should be noted that while cAMP was observed to have a stimulatory effect on C/EBPβ mRNA levels, no alteration in protein levels resulted (Figs. 1 and 2). This is not the first observation of a lack of correlation between C/EBPβ mRNA and protein abundance. Descombes et al. [27] examined the levels of rat C/EBPβ (originally termed LAP) mRNA and protein in different tissues, and observed that the ratio of protein/mRNA was almost 100 times higher in liver compared with lung, i. e. lung contains a high level of mRNA, little of which is translated into protein. Thus it appears that there may be a level of post-transcriptional regulation of this gene which modulates the amount of protein synthesized from the mRNA produced, an idea that gains further support from the present data.

One of the most significant findings of the present study is that the expression of C/EBPα is decreased in streptozotocin (STZ) diabetic rat liver. To our knowledge this is only the second transcription factor shown to be altered in expression as a result of diabetes, the other being hepatic nuclear factor-1 [28]. What is even more intriguing about our results relates to the fact that C/EBPα mRNA can be translated into several different protein products through selection of alternate initiation sites [18, 19]. While only the 42 kDa product was detected in our Western analysis of hepatoma extracts, both the 42 kDa and 29 kDa products are present in near equivalent amounts in rat liver (Fig. 4B). While the C/EBPα mRNA levels were decreased in STZ-diabetic liver, the translation of the 29 kDa form was particularly affected in comparison to the 42 kDa form, i. e. there was a shift towards preferential recognition of the first AUG.

This observation may be particularly important when considering the functional consequence of these changes. Structure/function analysis of C/EBPα by several groups have confirmed the critical role that the amino terminus plays in the transactivating ability of this transcription factor [29, 30]. Since the 29 kDa form of C/EBPα lacks the N-terminal 117 residues, this transcription factor is functionally inactive. Moreover, since it still possesses its DNA-binding and dimerization motifs, the 29 kDa form is able to dimerize with active 42 kDa forms, in the process forming inactive heterodimers. Thus, while the total amount of C/EBPα protein in the cell is decreased in STZ-diabetic liver, there may actually be an increase in the overall C/EBPα transactivating activity in diabetes due to a significant increase in the ratio of active compared to inactive forms. It should be noted that changes in the initiation site selectivity of C/EBPα mRNA has been previously observed under acute phase response conditions [31].

While the ultimate goal is to use this information on alterations in C/EBPα expression to make predictions and/or explain in molecular terms the basis for specific alterations in metabolic or physiological parameters that occur in STZ-diabetes, the complexity of most promoters makes this difficult because most changes are likely to be subtle. For example, C/EBPα plays an important role in the expression and regulation of the PEPCK promoter, participating in constitutive promoter activity as well as being a critical participant in cAMP responsiveness [3]. However, in addition to the three C/EBP binding sites in the 500 bp of 5' flanking region of this promotor, it also has binding sites for CREB, nuclear factor-1, hepatic nuclear factors-1, -3, -4, activator protein-1, and the thyroid hormone and glucocorticoid receptors [reviewed in 32]. Another example, the albumin promoter, contains not only a C/EBP binding site which is critical for its liver-specific expression, but also a minimum of three additional transcription factor binding sites which regulate its activity [33, 34]. The presence of these other transcription factors could somewhat buffer the impact of any change in C/EBPα expression on the transcriptional activity of an individual promoter, although some subtle yet chronic changes in constitutive or regulated expression of specific genes would likely occur that could lead to metabolic perturbations.

While we cannot make any specific comments on the physiological significance of an altered hepatic C/EBPα expression in STZ-diabetes, there is ample evidence to indicate that there would be metabolic and physiological consequences. C/EBPα knockout mice store no liver glycogen, have reduced expression of liver glycogen synthase, display developmentally delayed expression of hepatic PEPCK and glucose 6-phosphatase, and die from hypoglycaemia within 8 h of birth [35]. Our laboratory has also demonstrated that inhibition of C/EBPα in human hepatoma cells

results in a significant decrease in basal and cAMP-activated PEPCK gene transcription [3]. Thus C/EBPα appears to be a critical regulator of gluconeogenesis and glycogen balance, and an alteration in its level of expression is predicted to have metabolic consequences.

Valera et al. [36] previously reported that induction of STZ-diabetes resulted in a small increase in C/EBPα mRNA, in contradiction to our results. It is possible that some of the inconsistencies could be due to the differences in STZ dose used to induce diabetes, the duration and/or severity of diabetes, etc. It could also be due to the method of RNA analysis, with Valera et al. [36] using Northern blot analysis while we employed a relatively more sensitive RNase protection assay. It should also be noted that in the previous study, no assessment of C/EBPα protein was performed, whereas in the present study, we were able to extend and corroborate our mRNA analysis by measuring and observing corresponding changes in protein levels.

In summary, we have presented evidence indicating that the expression of C/EBPα is regulated in a complex hormonal fashion, and in an altered metabolic state as represented by diabetes. This regulation involves not only modulating the abundance of mRNA, but in some cases, regulating the relative production of active and inactive forms of the transcription factors. Given the large number of genes which are regulated by C/EBPα, the hormonal and metabolic regulation of its abundance should have a profound impact on a wide variety of gene products, such as enzymes, which are involved in energy homeostasis.

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