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Isolation and characterization of the promoter for the gene coding for the 68 kDa carnitine palmitoyltransferase from the rat

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Carnitine palmitoyltransferase (CPT) regulates the flux of long-chain fatty acids into the mitochondria for subsequent β-oxidation. A 485 bp segment of the promoter for the gene encoding the 68 kDa CPT was isolated from a rat λDASH genomic library using the polymerase chain reaction. The promoter contained a consensus binding sequence for CREB (cyclic AMP response element binding protein) at −153 to −166, and for C/EBPα (CCAAT/enhancer binding protein) at −115 to −128. DNAase I footprinting using proteins isolated from rat liver nuclei indicated the presence of several regions of nuclear protein binding, most notably at −95 to −130, at −273 to −295, and at a wide region encompassing −395 to −465. DNAase I footprinting studies with purified CREB and C/EBPα confirmed that protein binding to DNA occurred at the sites predicted by the consensus sequences. The segment containing 481 bp of 5′ flanking sequence plus 181 bp of untranslated mRNA was ligated to the structural gene for chloramphenicol acetyltransferase (CAT). When this plasmid was transfected into Hep G2 cells, CAT activity was stimulated 7-fold by addition of 1 mM-8-bromo-cyclic AMP (8-Br-cAMP) or co-transfection of the expression vector coding for the catalytic subunit of protein kinase A (PKA). The ability of several known second messengers and transcription factors to stimulate transcription of 68 kDa CPT promoter–CAT reporter was tested in co-transfection experiments. 68 kDa CPT promoter–CAT reporter transcription activity was stimulated 7-fold by addition of 8-Br-cAMP, and this induction was depressed 50% by the addition of phorbol esters. When the 68 kDa CPT promoter–CAT reporter was co-transfected with an expression vector for CREB or C/EBPα, transcription was increased 3- and 10-fold respectively. 8-Br-cAMP caused an additional 8-fold induction in the presence of each factor to yield 25- and 80-fold induction respectively. Co-transfection of the expression vector for c-*jun* also increased the CAT activity driven by the 68 kDa CPT promoter, while co-transfection with the expression vector for c-*fos* had no effect. When expression vectors for both c-*jun* and c-*fos* were co-transfected with the 68 kDa CPT promoter, c-*fos* depressed the induction seen with c-*jun* alone.

INTRODUCTION

Carnitine palmitoyltransferase (CPT; EC 2.3.1.23) regulates the flux of long-chain fatty acids into the mitochondria for subsequent β-oxidation (McGarry & Foster, 1980). The number of CPT proteins or subunits and their structure–function relationship within the mitochondrial outer and/or inner membranes have not been fully defined, complicating efforts to correlate CPT activity in various physiological and pharmacological states with changes in CPT gene expression. However, a 68 kDa CPT protein with CPT catalytic activity has been purified from isolated liver or heart mitochondria (Clarke & Bieber, 1981; Miyazawa *et al.*, 1983; Ozasa *et al.*, 1983; Brady & Brady, 1987; Woeltje *et al.* 1987; Ramsay, 1988). The cDNA for the 68 kDa CPT from liver has been cloned (Brady *et al.*, 1988; Woeltje *et al.*, 1990*a*) and sequenced (Woeltje *et al.*, 1990*a*). This CPT corresponds to, at least, the inner 68 kDa CPT activity.

Studies of the hormonal and dietary regulation of CPT (Brady *et al.*, 1989; Woeltje *et al.*, 1990*b*) indicate that the transcriptional regulation of the 68 kDa CPT is an important component in regulating CPT activity. Here we report the isolation of a 485 bp segment of the 68 kDa CPT promoter and demonstrate its regulation by specific transcription factors [cyclic AMP-responsive binding protein (CREB), CCAAT/enhancer binding protein (C/EBP), c-*jun*] known to modulate the expression of several other genes coding for hepatic enzymes involved in energy metabolism, and have evaluated the interaction with hormones that control the level of 68 kDa CPT in mitochondria.

EXPERIMENTAL

Materials

8-Bromo cyclic AMP (8-Br-cAMP) was obtained from Sigma, St. Louis, MO, U.S.A. All restriction enzymes were obtained from either Boehringer–Mannheim (Indianapolis, IN, U.S.A.) or Stratagene (La Jolla, CA, U.S.A.). The λDASH rat genomic library was purchased from Stratagene. [^{3}H]Chloramphenicol, [^{35}S]dATP, [^{35}S]dCTP and [^{32}P]dATP were obtained from DuPont New England Nuclear, Boston, MA, U.S.A. Reagents for the polymerase chain reaction were purchased as a kit (GeneAmp) from Perkin–Elmer, Norwalk, CT, U.S.A.

Abbreviations used: CPT, mitochondrial carnitine palmitoyltransferase; PEPCK, phosphoenolpyruvate carboxykinase; CREB, cyclic AMP-response element binding protein; C/EBP, CCAAT/enhancer binding protein; PKA, protein kinase A catalytic subunit; PKC, protein kinase C; C/EBPβ, liver-activating protein; cAMP, cyclic AMP; 8-Br-cAMP, 8-bromo cAMP; CAT, chloramphenicol acetyltransferase; PMA, phorbol 12-myristate 13-acetate; RSV, Rous sarcoma virus; IL-6-DBP, interleukin-6-dependent binding protein.

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Plasmids and probes

Oligonucleotides were synthesized by National Bioscience, Inc., Hamel, MN, U.S.A. pBluescript was obtained from Stratagene. The pTZ18RCAT plasmid containing the chloramphenicol acetyltransferase (CAT) structural gene driven by the phosphoenolpyruvate carboxykinase (PEPCK) promoter has been previously described (Mead *et al.*, 1986; Liu *et al.*, 1991). The PEPCK promoter was removed from the plasmid using *Bgl*II and *Xba*I, and the CPT promoter regulatory sequences were inserted into this site. The mammalian expression vectors for C/EBP were obtained from Dr. Steven McKnight, Carnegie Institute, Baltimore, MD, U.S.A., and contained the structural gene for C/EBP linked to the murine sarcoma virus promoter (Friedman *et al.*, 1989). The β-galactosidase vector was obtained from Chen Fang, Harvard University, Boston, MA, U.S.A. The CREB vector was a gift from Marc Montminy, Salk Institute, La Jolla, CA, U.S.A., and was prepared as described (Park *et al.*, 1990), while the vector for the protein kinase A catalytic subunit (PKA) was obtained from Dr. M. Muramatsu, DNAX, Palo Alto, CA, U.S.A. Expression vectors for c-*fos* and c-*jun* were obtained from Dr. Thomas Curran, Roche Institute of Molecular Biology, Nutley, NJ, U.S.A.

Isolation and sequencing of the promoter for the 68 kDa CPT

The 68 kDa CPT promoter was isolated from the λ DASH genomic library using a PCR-based approach. Two primers were synthesized and used in the PCR reaction; the downstream primer contained bases for the 5′ end of the 68 kDa CPT cDNA. In addition, a *Bgl*II restriction site was added to facilitate insertion into the *Bgl*II site of the pTZ18RCAT, and the *Bam*HI restriction site was added to allow insertion into the *Bgl*II site of pTZ18RCAT and the *Bam* HI site of the pBluescript (5′-ACCTGAGATCTCAGGACAAGCGAGGGGCAGCGGGGCCAGGCACG). The upstream primer was based on the sequence of the λDASH vector T3 promoter region and contained an *Xba*I site to facilitate oriented insertion into the *Xba*I sites of both vectors (5′-TTGCTAGCAGATCTAGAGTCGACAATTAACCCTCACTAAAGGGAACGAATTCGGAT C).Variations in the PCR conditions allowed the isolation of two putative 68 kDa CPT promoters. A 665 bp fragment was generated by cycling for 1 min at 94 °C, 1.5 min at 50 °C and 2 min at 72 °C for 30 cycles, with a final elongation cycle at 72 °C for 7 min. A 1815 bp fragment was generated by changing the conditions to 15 s at 94 °C, 30 s at 50 °C and 2 min at 72 °C for 30 cycles, with the same final elongation. This longer fragment represents additional sequence 5′ to the terminus of the shorter promoter. The sequence for the promoter fragment containing 665 bp (+181 to −485) is presented in Fig. 1(*a*).

Sequencing and transcription start site

The 68 kDa CPT promoter was digested with *Bgl*II and *Xba*I and inserted into the *Bam*HI/*Xba*I sites of pBluescript SK(−). The plasmid was sequenced from both ends as double-stranded DNA using a Taq-polymerase-based dideoxy method (*FastTaq*; IBI, New Haven, CT. U.S.A.). The transcription start site was determined by S1 nuclease analysis as follows (Ausubel *et al.*, 1987). First, the 68 kDa CPT promoter/pTZ18RCAT was digested with *Bgl*II and the 5′ phosphate was removed by digestion with calf intestinal alkaline phosphatase for 16 h. The 5′ ends were labelled with [γ-^{32}P]ATP. The labelled promoter/plasmid was then digested with *Xba*I and the labelled promoter was separated from the labelled plasmid by electrophoresis in a non-denaturing PAGE gel (0.4 mm thickness) (Ausubel *et al.*, 1987). The bands were visualized by exposing the wet gel covered with plastic film (Saran Wrap) to X-ray film for 3 min. The band corresponding to the 5′-end-labelled antisense promoter was cut from the gel and used for subsequent analysis. This probe was hybridized to mRNA and digested with S1 nuclease. The mass of the products was determined by urea/PAGE and comparison with [γ-^{32}P]ATP-labelled pBR322 fragments generated by *Hpa*II digestion.

Cell culture and transfection assay

Hep G2 human hepatoma cells were grown in Ham's F12/Dulbecco's modified Eagle's medium (1:1, v/v) supplemented with 5% fetal calf serum and 5% calf serum. DNA was transfected into the cells using calcium phosphate precipitation as previously described (Park *et al.*, 1990). The 68 kDa CPT promoter–CAT reporter plasmid was added to the cells at 10–20 μg/plate, and PKA, C/EBPα, C/EBPβ and CREB were each present at 5 μg/plate. Roux sarcoma virus promoter–β-galactosidase structural gene (1–2.5 μg) was added to each plate to serve as a control for transfection efficiency (MacGregor *et al.*, 1987). Hormones or 8-Br-cAMP were added 12–16 h before the cells were harvested and the activity of CAT was determined by the xylene phase extraction procedure as described (Ausubel *et al.*, 1987). The CAT activity for each sample was corrected based on the β-galactosidase activity in each individual cell extract.

DNA binding assay

Protein was extracted from rat liver nuclei as described by Gorski *et al.* (1986). The 68 kDa CPT promoter–CAT reporter vector was first digested with *Xba*I and the terminal phosphate was removed by treatment with calf intestinal alkaline phosphatase. The linearized plasmid was then end-labelled with [γ-^{32}P]ATP and T4 polynucleotide kinase. This was followed by digestion with *Bgl*II and purification on a 6% non-denaturing polyacrylamide gel. For DNAase footprinting, 2×10^4 d.p.m. of labelled DNA was used for each reaction. A 43 kDa recombinant partially purified C/EBPα was prepared from *Escherichia coli* as described by Landschulz *et al.* (1988). A 43 kDa recombinant CREB was obtained from Park *et al.* (1990). A 11 kDa fragment of C/EBPβ, which contains the leucine zipper and DNA binding domains, was obtained from Z. Cao, (Cao *et al.*, 1991).

RESULTS

Isolation and sequencing of the promoter for 68 kDa CPT

The sequence of the segment of the 68 kDa CPT promoter starting at −485 bp and consensus regulatory elements are presented in Fig. 1(*a*). We are aware that differences exist between our data and those of Woeltje *et al.* (1990*a*) with respect to the putative *N*-terminal start site. At present, we have no satisfactory explanation for this discrepancy. S1 analysis (Fig. 1*b*) did indicate two transcription start sites (labelled 1* and 2* in Fig. 1*a*). However, no recognized stop codon was apparent before the 1* site.

Identification of DNA binding sites

DNAase 1 footprinting analysis of the −485 bp of the 5′ flanking sequence of the 68 kDa CPT promoter starting at −485 was conducted using either proteins isolated from rat liver nuclei or recombinant proteins prepared from *E. coli* (Fig. 2). Three regions were protected by nuclear proteins from rat liver, a region from −100 to −130, a region from −273 to −295, and a more 5′ region from −407 to −455. The two members of the C/EBP family bound to the same sites on the promoter, with a higher affinity for the −115 to −128 sequence and a lower affinity for the −255 to −264 region. Finally, CREB bound to the sequence from −153 to −166.

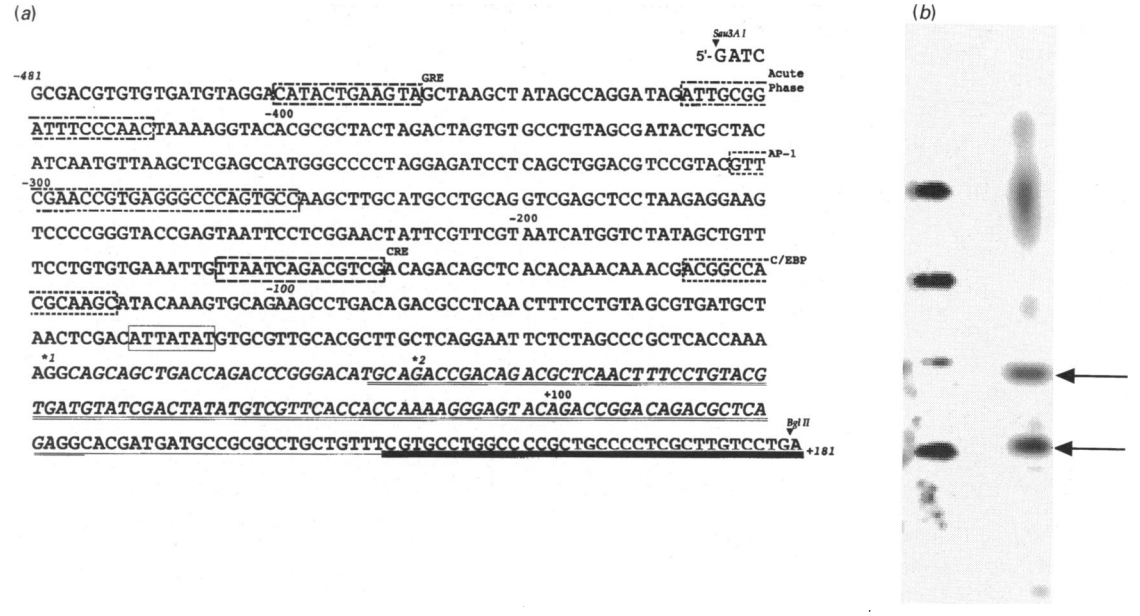

Fig. 1. Nucleotide sequence and transcription start site of the rat 68 kDa CPT promoter (−485 to +181)

(*a*) Sequence which also contains the 5′ segment of the cDNA for 68 kDa CPT reported by Woeltje *et al.* (1990*a*) and the 95 bp that we have previously found to match the sequence of the 5′ terminus of our cDNA clone. Two potential transcription start sites were identified and are designated by *1 and *2. The solid underline represents the region of agreement with Woeltje *et al.* (1990*a*); the double underline represents the 5′ region that we originally reported in our cDNA clone (Brady *et al.*, 1988). The TATA box is indicated by the solid box. A consensus sequence for C/EBPα is indicated by the short dashed box, while a consensus sequence for cAMP response element (CREB) is indicated by the long dashed box. A putative glucocorticoid response element and an acute phase protein response element are also indicated. (*b*) Transcription start site determined by S1 nuclease analysis. The 5′ terminus of the antisense strand of the 68 kDa CPT promoter was specifically labelled with [γ-^{32}P]ATP and hybridized to rat liver mRNA prior to S1 nuclease digestion. Fragments were resolved on a 0.4 mm sequencing gel (8 M urea/6% polyacrylamide). A [γ-^{32}P]ATP-labelled *Hpa*II digest of pBR322 was used as standard (right lane). Two potential start sites are indicated by arrows, corresponding to *1 and *2 of the sequence.

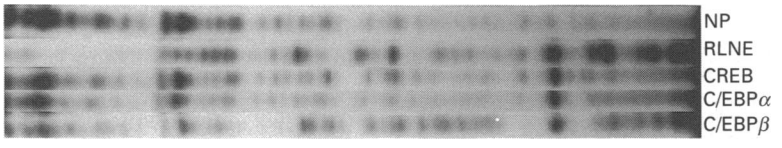

Fig. 2. DNA footprint of the rat 68 kDa CPT promoter using rat liver nuclear extract and various known transcription factors

DNAase footprinting was performed as described in the Experimental section. NP, no protein addition; RLNE, proteins isolated from rat liver nuclei extract; C/EBPβ indicates addition of purified C/EBPβ (or IL6-DBP). The right of the gel corresponds to +181.

Table 1. CPT–CAT activity in response to 8-Br-cAMP and PMA addition, and co-transfection of the expression vector for PKA

CPT–CAT and RSV–β-galactosidase were transfected into Hep G2 cells as described in the Experimental section. 8-Br-cAMP (1 mM) or PMA (1 μM) was added 12 h prior to harvest. PKA was co-transfected with CPT–CAT. Values are expressed as the CAT activity relative to the mean control value (CPT–CAT only; = 1.0). Results are means ± S.E.M. of five values.

	Relative CAT activity		
	No addition	8-Br-cAMP (1 mM)	PKA
No addition	1.0±0.1	7.3±1.5	8.0±1.0
PMA (1 μM)	1.6±1.0	2.8±0.8	4.1±1.2

Functional response of the promoter for 68 kDa CPT

Previous data showed that both transcription of the 68 kDa CPT gene and the level of 68 kDa CPT mRNA were induced by 1 mM-cyclic AMP (cAMP) in H4IIE hepatoma cells (Wang *et al.*, 1989). Since H4IIE cells are difficult to transfect, we transfected Hep G2 cells with the 485 bp 68 kDa CPT promoter–CAT reporter chimaeric gene to study the functional activity of the 68 kDa CPT promoter (Park *et al.*, 1990). The segment of the 68 kDa CPT promoter starting at −485 bp described above was regulated by 1 mM-8-Br-cAMP and by PKA. Addition of 8-Br-cAMP or co-transfection of the structural gene for PKA caused a 7-fold increase in transcription from the 68 kDa CPT promoter, as indicated by CAT activity in Hep G2 cells. The response of the 1815 bp segment of the 68 kDa CPT promoter was identical. Insulin (1 μM) had no effect upon transcription from the 68 kDa CPT promoter (as reflected by CAT activity), either when added alone or with 8-Br-cAMP (results not shown). Phorbol myristate acetate (PMA) addition (1 μM) to cells transfected with the 68 kDa CPT–CAT gene had little effect on 68 kDa CPT promoter activity (Table 1). However, PMA added either in addition to 8-Br-cAMP or to cells that were co-transfected with PKA caused a 50% decrease in the effects of 8-Br-cAMP or PKA. When the expression vector for PKC was co-transfected with 68 kDa CPT–CAT or the combination of 68 kDa CPT–CAT + 8-Br-cAMP or 68 kDa CPT–CAT and the catalytic subunit of PKA, the stimulatory effects of these treatments were depressed (results not shown).

Table 2. CPT–CAT activity in response to co-transfection with the expression vectors RSV–CREB, murine sarcoma virus (MSV)–C/EBPα and MSV–C/EBPβ with and without addition of 8-Br-cAMP or the expression vector for PKA

HepG2 cells were transfected as described in the Experimental section. Values are expressed as the CAT activity relative to the average control value (CPT–CAT only) value. Results are means ± S.E.M. of five values.

	Relative CAT activity		
	CREB	C/EBPα	C/EBPβ
No addition	3.2 ± 1.5	9.8 ± 2.0	8.7 ± 2.0
8-Br-cAMP (1 mM)	18.5 ± 3.2	79.1 ± 15.1	28.4 ± 5.0
PKA	19.0 ± 3.8	80.0 ± 14.0	31.5 ± 4.5

Since the 68 kDa CPT promoter can bind both CREB and members of the C/EBP family, we next examined whether CREB or C/EBP could transactivate the 68 kDa CPT promoter. An expression vector for CREB or C/EBPα (Friedman *et al.*, 1989) was co-transfected with 68 kDa CPT–CAT into Hep G2 cells (Table 2). Transcription from the 68 kDa CPT promoter was increased 10-fold. Similar experiments with expression vectors for C/EBPβ (Cao *et al.*, 1992) or CREB led to 7-fold and 4-fold inductions respectively (Table 2). In order to examine whether over-expression of any of these factors enhanced the cAMP responsiveness of the 68 kDa CPT promoter, we added cAMP or the expression vector for PKA in the co-transfection experiment (Table 2). Addition of cAMP or the PKA vector increased the induction by C/EBPα from 10- to 80-fold. The enhancement by C/EBPβ was raised from 7- to 25-fold. The 4-fold CREB induction was increased to 16-fold by cAMP or the PKA expression vector.

We also examined the effects on the 68 kDa CPT promoter of the proto-oncogenes c-*jun* and c-*fos*, whose levels can rise in response to a variety of stimuli. Addition of the expression vector for c-*jun* increased transcription activity 50 ± 8-fold over control (mean ± S.E.M. five observations), while co-transfection with the expression vector for c-*fos* produced little, if any, effect (2.5 ± 1.0-fold increase). Transfection of c-*fos* in combination with c-*jun* depressed (22 ± 5-fold) the transcriptional activity seen with c-*jun* alone. Thus it appeared that c-*fos* had an inhibitory effect on the induction of transcription from the 68 kDa CPT promoter caused by c-*jun*.

DISCUSSION

In the present paper, we have extended the knowledge of regulation of the 68 kDa CPT to include a basic understanding of transcriptional responses to physiological signals. The 68 kDa CPT promoter responds to cAMP and various nuclear regulatory proteins that have been shown to regulate other promoters and which may be intermediates in signal transduction pathways that regulate metabolism.

Using PCR-based approaches, we were able to obtain two promoter segments, one of approx. 1815 bp and the second of 665 bp (−485 to +181). Both promoter segments were regulated similarly (see the Results section). We have also isolated a 2000 bp promoter for the gene encoding human 68 kDa CPT and have found that it also is induced by cAMP and the expression vectors for CREB, C/EBPα and C/EBPβ in co-transfection experiments, although its sequence varies from that of the rat (P. S. Brady, E. A. Park, R. W. Hanson & L. J. Brady, unpublished work). The 68 kDa CPT promoter from the rat contains a CREB site (−153 to −166) and a single high-affinity C/EBP binding site (−115 to −128). This contrasts with the PEPCK gene, which has similar hormonal regulation, but where both CREB and C/EBP can bind the CRE-1 sequence. The C/EBP site of the 68 kDa CPT promoter overlapped with regions protected by proteins isolated from rat liver nuclei extract, which in combination with the potent ability of C/EBP to transactivate the promoter, suggests that this protein may be important in directing hepatic 68 kDa CPT expression. Promoters of the genes for serum albumin (Friedman *et al.*, 1989) and stearoyl-CoA desaturase (Christy *et al.*, 1989) also contain similar C/EBPα response elements. While the more 3′ regions of the 68 kDa CPT promoter were protected by either proteins from a rat liver nuclear extract or purified proteins (CREB, C/EBPα, C/EBPβ) one upstream region (−395 to −465) was protected only by the rat liver nuclear extract.

We have previously shown that 68 kDa CPT gene expression was increased by cAMP and inhibited by insulin (Wang *et al.*, 1989). In the present studies, we have shown that 8-Br-cAMP or the catalytic subunit of PKA stimulated transcription from the 68 kDa CPT promoter. This response could be mediated by various nuclear transcription factors, as has been shown for other promoter elements such as those for PEPCK and human chorionic gonadotrophin (Roesler *et al.*, 1988, 1989; Liu *et al.*, 1991). In many genes, an important factor in the response to cAMP is CREB, which is phosphorylated on serine residues by PKA. The phosphorylation of CREB is critical for transcriptional activation of the somatostatin gene (Gonzalez & Montminy, 1989). Liu *et al.* (1991) found that CREB had little effect on PEPCK–CAT transcription, but that co-transfection with PKA plus CREB increased transcription from the PEPCK promoter 80-fold. Recent evidence suggests that the ATF/CREB and *fos.jun* families of nuclear proteins might form heterodimers that bind to DNA with differing affinities and at different sites (Hai & Curran, 1991). A common feature of promoters that contain both ATF/CREB and AP-1 binding sites is that they respond to environmental stimuli. The 68 kDa CPT promoter contains consensus response elements to both AP-1 and CREB. c-*fos*, c-*jun* and CREB may represent the nuclear component of signal transduction from various hormones and pharmaceutical agents which have been shown to increase 68 kDa CPT gene expression. Our data do not clarify which proteins or sequences are involved in the cAMP induction of 68 kDa CPT transcription. However, different combinations of factors may transmit the cAMP responsiveness in different tissues. Schilling *et al.* (1991) have recently shown that the c-*fos* promoter responds to α and β adrenergic agents, cAMP and phorbol ester in neuroblastoma cells. These agents also increase 68 kDa CPT gene expression. The high level of transactivation of the 68 kDa CPT promoter by CREB and c-*jun* suggests that it falls within the group of enzymes of energy metabolism that are critically regulated by the interaction of specific nuclear factors.

C/EBPα is distributed mainly in liver, intestine, adipose tissue, lung, adrenal gland and placenta (Birkenmeier *et al.*, 1989). In liver and intestine, C/EBPα is induced just prior to birth. McKnight's group has suggested that C/EBPα might be a significant regulator of energy metabolism in differentiated cells, especially those such as in liver, adipose tissue, and lung which metabolize large amounts of lipid substrate (Birkenmeier *et al.*, 1989; McKnight *et al.*, 1989). These conclusions were based on the developmental pattern of C/EBPα and its correlation with enzymes of intermediary metabolism such as PEPCK and glycerol phosphate dehydrogenase. Umek *et al.* (1991) have also suggested that C/EBPα promotes terminal differentiation of adipocytes and might regulate the balance between cell growth and differentiation. Christy *et al.* (1989) have characterized the murine

C/EBPα promoter and shown that its transcription is increased prior to that of several adipose-specific genes whose promoters are transactivated by C/EBPα. Ro & Roncari (1991) have shown that the C/EBPα binding site and its binding factors may serve as negative regulators in undifferentiated human preadipocytes. In the present paper we have shown that C/EBPα is capable of binding to and transactivating the CPT promoter. The developmental profile of 68 kDa CPT is similar to that of C/EBPα in liver. Since 68 kDa CPT is a central regulatory enzyme in lipid metabolism, our work provides further support for the suggestion that C/EBPα plays an important role in regulating energy metabolism. However, multiple factors will be involved in 68 kDa CPT regulation, since it is also highly expressed in cardiac and skeletal muscle. Interestingly, C/EBPβ and C/EBPα had similar effects on transcription from the 68 kDa CPT promoter. C/EBPβ mRNA accumulated in liver, lung, spleen, kidney, brain and testis, but was highly enriched in lung. In contrast, C/EBPβ protein accumulated to the greatest extent in liver, indicating post-transcriptional regulation (Descombes *et al.*, 1990). C/EBPβ bound to the same promoter elements of the albumin gene as did C/EBPα, and was able to transactivate (50–150-fold induction) a chimaeric gene containing the albumin promoter linked to a CAT reporter gene in Hep G2 cells.

Although it is known that insulin depresses the stimulatory effects of cAMP on the endogenous 68 kDa CPT gene (Wang *et al.*, 1989), we were unable to demonstrate a negative effect of insulin on transcription from the 68 kDa CPT promoter in the Hep G2 cells. The lack of response to insulin in a stably transfected system has been noted before with the PEPCK promoter–CAT reporter (Wynshaw-Boris *et al.*, 1986), as well as in transiently transfected systems (E. A. Park & R. W. Hanson, unpublished work). However, subsequent reports found an insulin responsive element in the PEPCK promoter in stable transfectants (O'Brien *et al.*, 1990; Forest *et al.*, 1990). It is possible that the 481 bp segment of the 68 kDa CPT promoter does not contain the insulin response element.

In the present paper, we have characterized the promoter elements for 68 kDa CPT that presumably modulate responses *in vivo* to hormones and drugs. The continuing challenge will be to identify the specific transcription factors that modulate the 68 kDa CPT promoter as the physiological state changes. Understanding the mechanism of 68 kDa CPT regulation will ultimately require identification of all of the nuclear proteins which bind to this promoter and how they interact. Preliminary evidence in septic rats, using gel mobility shift analysis, suggests that there are qualitative and quantitative changes in the rat liver nuclear proteins binding to the AP-1, CRE and acute phase sites (R. A. Barke, P. S. Brady & L. J. Brady, unpublished work). The acute phase sites includes an interleukin-6-dependent binding protein (IL-6-DBP) binding domain. Of considerable interest, Akira *et al.* (1990) and Poli *et al.* (1990) demonstrated that the IL-6-DBP was C/EBPβ. Identification of additional specific nuclear proteins induced in sepsis, and definition of their interaction with DNA response elements, should provide insight both into the changes in transcription seen in sepsis but also into the interaction of nuclear proteins with multiple DNA response elements of the 68 kDa CPT promoter.

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