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The Coactivator PGC-1 Is Involved in the Regulation of the Liver Carnitine Palmitoyltransferase I Gene Expression by cAMP in Combination with HNF4 α and cAMP-response Element-binding Protein (CREB)*

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Liver carnitine palmitoyltransferase I catalyzes the transfer of long-chain fatty acids into mitochondria. L-CPT I is considered the rate-controlling enzyme in fatty acid oxidation. Expression of the L-CPT I gene is induced by starvation in response to glucagon secretion from the pancreas, an effect mediated by cAMP. Here, the molecular mechanisms underlying the induction of L-CPT I gene expression by cAMP were characterized. We demonstrate that the cAMP response unit of the L-CPT I gene is composed of a cAMP-response element motif and a DR1 sequence located 3 kb upstream of the transcription start site. Our data strongly suggest that the coactivator PGC-1 is involved in the regulation of this gene expression by cAMP in combination with HNF4 α and cAMP-response element-binding protein (CREB). Indeed, (i) cotransfection of CREB or HNF4 α dominant negative mutants completely abolishes the effect of cAMP on the L-CPT I promoter, and (ii) the cAMP-responsive unit binds HNF4 α and CREB through the DR1 and the cAMP-response element sequences, respectively. Moreover, cotransfection of PGC-1 strongly activates the L-CPT I promoter through HNF4 α bound at the DR1 element. Finally, we show that the transcriptional induction of the PGC-1 gene by glucagon through cAMP in hepatocytes precedes that of L-CPT-1. In addition to the key role that PGC-1 plays in glucose homeostasis, it may also be critical for lipid homeostasis. Taken together these observations suggest that PGC-1 acts to coordinate the process of metabolic adaptation in the liver.

Liver mitochondrial long-chain fatty acid oxidation and ketogenesis are closely related to the appearance of the carnitine palmitoyltransferase (CPT)¹ system. The CPT system, which

controls the transfer of long-chain fatty acids inside the mitochondria, is composed of three distinct entities: CPT I localized in the outer mitochondrial membrane, carnitine-acylcarnitine translocase and CPT II localized on the inner mitochondrial membrane (1). It is generally accepted that CPT I is the rate-limiting enzyme for the oxidation of long-chain fatty acids in the liver (1). Two isoforms of CPT I have been cloned (2, 3). The "liver" isoform (L-CPT I) is expressed in most tissues including liver, kidney, and pancreatic β cells (2) while the "muscle" isoform (M-CPT I) is expressed in heart, skeletal muscle, and adipose tissue (3, 4).

Expression of the CPT I gene in liver is regulated during development and by several hormones and nutrients (1). At birth, rat hepatic CPT I mRNA increases, remains high during the suckling period, and then decreases after weaning onto a high carbohydrate diet (5). Few studies have addressed the direct effects of pancreatic hormones on the regulation of L-CPT I gene expression. However, it was shown that the addition of glucagon or its second messenger, cyclic AMP, induces transcription of the L-CPT I gene without modification of mRNA half-life (6). In cultured fetal rat hepatocytes, insulin antagonizes the cAMP-induced accumulation of CPT I mRNA (7). These observations suggest a prominent role for pancreatic hormones in the regulation of L-CPT I gene expression.

The rat L-CPT I gene has been cloned, and its promoter partially characterized (8). Basal expression of L-CPT I is driven by Sp1 and NF-Y transcription factors (9). Initial characterization of the DNA sequences involved in the hormonal and nutritional response of L-CPT I gene expression allowed the identification of: (i) a functional thyroid hormone (T3)-response element located approximately –3 kb from the transcription start site of the gene (10), (ii) a peroxisome proliferator-response element, located at –3 kb from the start site, corresponding to a classical DR1 element (11), and (iii) a fatty acid-response element present in the first intron of the gene (11).

Cyclic AMP activates eukaryotic gene transcription via binding of nuclear factors to cAMP-responsive elements (CRE). The classical CRE consists of a relatively well conserved 8-bp pal-

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This paper is dedicated to the memory of J. D. McGarry who passed away on January 27, 2002.

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¹ The abbreviations used are: CPT, carnitine palmitoyltransferase; L-CPT, liver CPT; CRE, cAMP response element; CREB, cAMP-re-

sponse element binding protein; CREM, cAMP-response element modulator; C/EBP, CAAT/enhancer binding protein; PKA, protein kinase A; CBP, CREB-binding protein; TAT, tyrosine aminotransferase; PEPCK, phosphoenolpyruvate carboxykinase; CRU, cAMP response unit; CAT, chloramphenicol acetyltransferase; EMSA, electrophoretic mobility shift assays; Hss, hypersensitive site; DR, direct repeat; HNF, hepatocyte nuclear factor.

indrome (TGACGTCA) (12). Nuclear factors involved in cAMP-responsive transcriptional activation include cAMP-response element binding protein (CREB), cAMP-response element modulator (CREM), activating transcription factor-1 (ATF-1), and potentially, members of the nuclear receptor superfamily (13). CREB proteins belong to a family of transcription factors that contain a basic region/leucine zipper (bZIP). The bZIP family includes Jun, Fos, CAAT/enhancer binding protein (C/EBP), and yeast GCN4 (13). Current models of cAMP-responsive transcriptional activation invoke phosphorylation of proteins of the CREB family on a single serine residue in response to activation of protein kinase A (PKA) by cAMP. Phosphorylation of CREB promotes the recruitment of the transcriptional coactivator CREB-binding protein (CBP) and increases cAMP-induced transcription. However, one major limitation of this model is that CREB, ATF-1, and CBP genes are all ubiquitously expressed and are not transcriptionally activated by cAMP (13). Thus, this model cannot explain the sensitivity of liver-specific genes such as tyrosine aminotransferase (TAT) and phosphoenolpyruvate carboxykinase (PEPCK) to cAMP. In this regard, the cAMP-dependent enhancement of TAT expression is dependent upon the cooperative action of CREB and HNF4 α , allowing tissue-specific control of this gene (14). Similarly, while the promoter of the PEPCK gene contains a classical CRE element, maximal cAMP induction of this gene requires multiple cis-acting sites within a so-called "cAMP response unit" (CRU) (15). The CRU of the PEPCK promoter contains five cis-elements including the CRE and four binding sites for a C/EBP (15). Therefore, the molecular mechanisms involved in the cAMP-mediated induction of target genes seem to be more complex than the simple presence of a CRE motif.

Recently, PPAR γ coactivator-1 (PGC-1) was shown to be involved in the regulation of gluconeogenic genes by cAMP and glucocorticoids (16). PGC-1 is strongly induced in mouse liver by fasting, insulin deficiency (e.g. streptozotocin-induced diabetes, ob/ob genotype, and liver insulin receptor knockout), and, in primary cultures of hepatocytes, by cAMP (16). Furthermore, both PEPCK and glucose-6-phosphatase genes are activated by overexpression of PGC-1 (16). Full transcriptional activation of the PEPCK promoter requires a coactivation of the glucocorticoid receptor and HNF4 α (16). These data strongly suggest that PGC-1 could be a key modulator of the metabolic adaptation of the liver.

Here, we have characterized a cAMP-responsive unit in the L-CPT I gene. The CRU contains two cis-acting DNA sequences: a CRE and a DR1 motif. We demonstrate that CREB and HNF4 α bind to the L-CPT I promoter via the CRE and DR1, respectively. Moreover, we show that the nuclear receptor coactivator PGC-1 strongly stimulates the L-CPT I promoter. In addition, PGC-1 expression is transcriptionally activated by cAMP and glucagon in primary cultures of rat hepatocytes. As this induction precedes that of L-CPT I, we suggest that PGC-1 is involved in the activation of the L-CPT I gene by cAMP. Taken together, our results suggest that PGC-1, in addition to controlling gluconeogenesis, also plays a role in the adaptation of hepatic lipid metabolism in response to nutritional stimuli.

EXPERIMENTAL PROCEDURES

Animals

Female Wistar rats bred in our laboratory were housed in plastic cages at a constant temperature, 24 °C, with light between 6.00–20.00 h. They had free access to water and chow pellets (65% carbohydrate, 11% fat, 24% protein by energy content). Livers from 21-day-old fetuses delivered by cesarean section and 1-day-old suckling newborn rats were used for nuclear extract isolation.

Isolation and Culture of Suckling Rat Hepatocytes

12-day-old suckling pups were anesthetized with pentobarbital (50 mg/kg). Hepatocytes were isolated by liver perfusion as described previously (17). After measurement of cell viability by trypan blue exclusion, hepatocytes were suspended in M199 medium (Invitrogen) containing 5.5 mM glucose, 0.1% (mass/v) bovine serum albumin, and 2% (v/v) Ultrosor G (Invitrogen). Cells (2×10^6) were plated in 2 ml of M199 medium in 12-well plates. Cultures were maintained at 37 °C and 5% CO $_2$. After plating for 4 h, the medium containing unattached cells was removed and replaced by 1 ml of fresh M199 without Ultrosor G or bovine serum albumin for the transfection experiments.

Plasmids Constructs

Reporter Constructs—The reporter plasmids CPT20, CPT18, CPT14, and the CPT15 contain 6870, 4495, 1653, and 193 bp, respectively, of the 5' flanking sequence from the transcription start site of the L-CPT I gene (8). A *Pst*I-*Eco*RI fragment (–3000 to –1653) from the original P1 clone (8) was ligated into the *Pst*I and *Eco*RI sites of pBS II K/S+ (Stratagene, La Jolla, CA). The *Sma*I-*Xho*I fragment from this vector was ligated into *Sma*I-*Xho*I of CPT15 to yield CPT16.

L-CPT I (–2967 to –2773) was described previously (11). Serial deletions of this construct were created by PCR. The 3' end deletion including L-CPT I (–2967 to –2831) and the L-CPT I (–2967 to –2871) constructs were made with the same forward primer including a *Hind*III restriction site: (–2967) 5'-TGAAGCTTGGGGTTTGTATCCTTG-3' and two different reverse primers containing *Xba*I restriction sites: (–2831) 5'-CCTCTAGACCTCAGTTCCCCCTGA and (–2871) 5'-CCTCTAGACGTTTTTGAGTCAATA-3'. L-CPT I (–2938 to –2831) was generated with the same reverse primer (–2831) and the forward primer (–2938): 5'-CGAAGCTTTCCTCATGGAACCT-3'. These *Hind*III-*Xba*I PCR fragments were ligated into the pBLCAT5 vector (18). Automated DNA sequencing confirmed that the nucleotide sequence of the PCR product was 100% identical to the template sequence. The heterologous construct containing the CRE sequence of the rat somatostatin promoter was described previously (19).

The CRE2x, DR1, CRE, and CRE/DR1 constructs were prepared by ligation of double-stranded oligonucleotides into the *Hind*III and *Xba*I sites of the pBLCAT5 vector. Oligonucleotides used for each construct were as follows: CRE2x-upper-5'-AGCTCCTGGTGACGCTGGCCCTGTGACGCTGGC-3', CRE2x-lower-5'-CTAGGCCAGCGTCACAGG-GCCAGCGTCACAGG-3', DR1-upper-5'-AGCTCTCAAAACGCTGTACAGGAGCTCAAAGTTCAAGTTCAAGTTCAAG-3', DR1-lower-5'-CTAGCCTGAACCTGAACTTTGAGCTCCTGTACACGTTTTGAG-3', CRE-upper-5'-AGCTGCTGTCTCATGGAACCTGGTGACGCTGGCTGAACAA-3', CRE-lower-5'-CTAGTTGTTCAGCCAGCGTCACAGGTTCCATGAGGACAGC-3', CRE/DR1-upper-5'-AGCTGGTGACGCTGGCACAGGAGCTCAAAGTTCAAGTTCAAG-3', and CRE/DR1-lower-5'-CTAGCCTGAACCTGAACTTTGAGCTCCTGTGCCAGCGTACC-3'. Site-directed mutagenesis was conducted using the *Dpn*I QuikChange method (Stratagene, La Jolla, CA). Oligonucleotides used to disrupt each DNA motif were: CREm-upper-5'-CATGGAACCTGGT-aAgcCTGGCTGAAC-3', AP1m-upper-ACCTCCGCTATTCTcGtAAAACGTGTACAGG-3', and DR1m-upper-CGTGTACAGGAGCTCAgcGaaC-AAGTTCAAGG-3'. Mutation in each construct (CREm, DR1m, AP1m, and CREm-DR1m) was confirmed by sequence analysis.

Expression Vector—The expression vector pSV-PKA-C α codes for the catalytic α subunit of PKA (20). The vector coding for Dn-HNF4 α is a selective dominant negative mutant that forms defective heterodimers with wild-type HNF4 α , preventing DNA binding and transcriptional activation by HNF4 α . It was a gift from T. Leff (21). The dominant negative mutant Dn-CREB that blocks binding of CREB and closely related transcription factors (such as ATF1) to DNA was a gift from D. Ginty (22). The expression vectors pSVSport-PGC-1 and VP16-CREB were described previously (16).

Transient Transfection Protocol

Primary cultures of rat hepatocytes were transfected as described by Boussif *et al.* (23). To increase transfection efficiency, 200 plaque-forming units/cell of Ad-RSV-nlsLacZ (Rous sarcoma virus promoter driving the nlsLacZ gene) were added simultaneously (24). Briefly, cells were transfected with 2.5 μ g of DNA plasmid per plate and polyethylenimine (0.56 μ l of polyethylenimine/ μ g of DNA) in serum free medium. In some experiments, cells were cotransfected with 1 μ g of expression vectors. Transfection was accomplished by dilution of polyethylenimine and plasmid in 25 μ l of 150 mM NaCl. They were mixed together, and the polycation/DNA complex was diluted into 1 ml of serum-free medium in 12-well culture dishes. After 7 h of transfection, the medium

was changed and Bt2cAMP at 10^{-4} M, glucagon at 10^{-8} M, or vehicle was added for 24 h (luciferase assay) or 48 h (CAT assay). Cells were lysed in reporter lysis buffer (Promega), harvested by scraping, and centrifuged at 8000 g at 4 °C for 5 min. Protein concentration, luciferase, and CAT activities were determined in the supernatant. Luciferase activity was assayed on 20 μ l of supernatant using the Promega kit (Promega). CAT activity was assayed as described by Seed and Sheen (25).

Chromatin Structure of the L-CPT I Promoter and Mapping of DNase I Hypersensitive Sites

Nuclei for chromatin studies were isolated from fresh tissues as described by Schibler *et al.* (26). Isolated nuclei were suspended in nuclear storage buffer: 0.3 M sucrose, 60 mM KCl, 15 mM NaCl, 0.1 mM EGTA, 0.2 mM EDTA, 0.15 mM spermine, 0.5 mM spermidine, 15 mM Tris/HCl, pH 7.5, 5% (v/v) glycerol, 0.5 mM dithiothreitol, and 0.1 mM phenylmethylsulfonyl fluoride. Frozen nuclei were thawed and resuspended at a DNA concentration of 2 mg/ml in digestion buffer containing 60 mM KCl, 0.1 mM EGTA, 5% (v/v) glycerol, 15 mM Tris/HCl, pH 7.5, and 0.5 mM dithiothreitol. Nuclei were prewarmed to 37 °C, and digestions initiated by adding nuclei into tubes containing 5 mM MgCl₂ and DNase I (Invitrogen) ranging over 1–8 μ g/ml. Control digestion tubes contain MgCl₂ but no DNase I. After 15 min of digestion at 37 °C, the reaction was stopped by addition of the same volume of a buffer containing 150 mM NaCl, 15 mM EDTA, 0.3% (mass/v) sodium dodecyl sulfate, and 50 mM Tris/HCl, pH 7.5. Nuclei were digested by RNase A (50 μ g/ml) for 1 h at 37 °C and then incubated overnight at 37 °C with proteinase K (100 μ g/ml). DNA was extracted and quantified by spectrophotometry.

For studies of DNase I hypersensitive sites, DNA from undigested and DNase I from digested nuclei was cut with *Eco*RI or *Hind*III (4 units/ μ g DNA) for 16 h at 37 °C, subjected to electrophoresis, and transferred onto Hybond N+ filter (Amersham Biosciences). For hybridization, two genomic probes were used: a 744-bp *Eco*RI-*Sph*I fragment localized at –1653 bp from the transcription start site named S1 and an 855-bp *Hind*III-*Bam*HI fragment located in the first intron named S2. Probes were labeled with [α -³²P]dATP using the multiprimer labeling system kit (Amersham Biosciences).

Total RNA Extraction and Analysis

Total RNA was extracted from cultured rat hepatocytes according to Chomczynski and Sacchi (27). Total RNA (15 μ g) was separated on 1% agarose gels containing 2.2 M formaldehyde and transferred to Hybond N membranes (Amersham Biosciences). Filters were hybridized overnight with ³²P-labeled cDNA probes. The PEPCK probe was previously described (28). L-CPT I and PGC-1 probes were amplified by PCR with the following primers: CPT I-upper-5'-ACCAAGCTGTGGCCTTCCAGTTCACCGTCAAC-3'; CPT I-lower-5'-GCGTGGCAGAGACGCTCTGGAA-GCTGTACAA-3'; PGC-1-upper-5'-TGATGGAGTGACATAGAGT-3'; and PGC-1-lower-5'-GCTAAGACCGCTGCATTCAT-3'.

Electrophoretic Mobility Shift Assays (EMSA)

In vitro translated HNF4 α was generated using the TnT T7-coupled reticulocyte lysate system (Promega). Recombinant HNF4 α and CREB proteins were prepared as described previously (29, 30). Nuclear protein extracts from liver and hepatocytes of rat were prepared with NE-PER kit (Pierce). *In vitro* translated proteins, recombinant HNF4 α , recombinant CREB, or nuclear protein extracts, were incubated at 4 °C for 30 min with 30,000 cpm of labeled double-stranded oligonucleotides in a buffer containing 20 mM Tris/HCl, pH 7.5, 100 mM KCl, 14% (v/v) glycerol, 2 mM dithiothreitol, and 1 μ g of poly(dI-dC) in a final volume of 20 μ l. In the supershift assay antibodies to HNF4 α or CREB were added to the binding reaction. Complexes were separated on a 6% (v/v) native polyacrylamide gel in 0.25 \times TBE running buffer at 200 V for 2 h. For DNA binding competition experiments, excess unlabeled double-stranded competitor oligonucleotide was added to the incubation reaction as detailed in the figure legends. Following electrophoresis gels were dried and exposed to x-ray films. The sense oligonucleotides used were: 5'-AGTCAAAAGTGACAGGAGCTCAAAGTTCAAGTTCAAG-3' for L-CPT I-DR1; 5'-AGCTGTGTCTCTCATGGAACCTGGTGAC-GCTGGCTGAACAA-3' for L-CPT I-CRE; and 5'-GATCGCCTCCTTG-GCTGACGTCAGAGA-3' for somatostatin-CRE.

L-CPT I expression in Liver-specific HNF4 α -null Mice (H4LivKO)

Liver-specific HNF4 α -null mice, in which disruption of a loxP-targeted allele of HNF4 α is driven by an albumin-Cre transgene, have been described (31). 45-day-old mice were anesthetized with 2.5% aver-

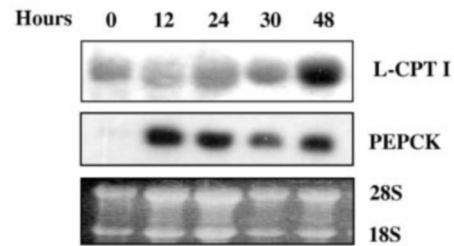


FIG. 1. Time course of cAMP-induced L-CPT I mRNA level in primary culture of hepatocytes. 12-day-old rat hepatocytes were cultured as described under "Experimental Procedures" for 12, 24, 30, and 48 h in the presence of Bt2cAMP (10^{-4} M). Total RNA was isolated, and the level of CPT I and PEPCK mRNAs was measured by Northern blot using specific radiolabeled probes. This blot is representative of two different experiments.

tin and killed by decapitation, and livers were collected and frozen immediately in liquid nitrogen. Total liver RNA was prepared as previously described (31). Poly A⁺ RNAs were prepared from total liver RNA using the Poly A Tract mRNA kit (Promega). Poly(A)⁺ RNAs (1 μ g) were separated on 1% agarose, 2.2 M formaldehyde gels prior to blotting to Hybond-N+ membranes (Amersham Biosciences). Blots were hybridized by using successively specific probes of L-CPT I and glyceraldehyde-3-phosphate dehydrogenase (31).

RESULTS

Identification of the CRU in the Rat L-CPT I Gene Promoter—We have shown that L-CPT I is transcriptionally activated by glucagon or its second messenger cAMP (7). To further characterize this effect, primary cultures of suckling rat hepatocytes were cultured in the presence or in the absence of cAMP. A significant induction of L-CPT I mRNA concentration was observed after 48 h exposure to cAMP (Fig. 1). In the same culture conditions, PEPCK mRNA was induced by cAMP after 12 h of culture (Fig. 1). Thus, the response of the L-CPT I gene to cAMP is delayed relative to that of PEPCK. This delayed response suggests that different mechanisms may control the induction of these two genes in response to cAMP.

To determine the DNA sequences involved in the cAMP induction of L-CPT I gene transcription, transient transfections were performed in primary culture of suckling rat hepatocytes. When the full-length promoter (–6870 to +19) of the rat L-CPT I gene was transfected, a specific increase of luciferase activity (2.7 ± 0.2 ; $n = 7$) was observed after cAMP exposure (Fig. 2). This induction was also observed using a shorter construct (–4830 to +19) suggesting that the 2-kb DNA sequence (from –6870 to –4830) was not involved in the activation of the L-CPT I gene by cAMP (Fig. 2). In contrast, this induction was abolished when a DNA sequence spanning from –4830 bp to –1654 bp was removed (Fig. 2). Therefore, DNA sequences between –4830 bp and –1654 bp are involved in the cAMP response of the L-CPT I gene.

To determine whether this DNA sequence was sufficient by itself to mediate the stimulatory effect of cAMP on the L-CPT I promoter, a construct spanning nucleotides from –3000 bp to –1654 bp linked to the minimal promoter of the L-CPT I gene was made. An increase in luciferase activity (2.2 ± 0.15 ; $n = 4$) was observed upon cAMP exposure by comparison to a construct containing the L-CPT I minimal promoter (Fig. 2). Thus, the L-CPT I gene contains a CRU between –3000 bp and –1654 bp.

To define the chromatin structure within the CRU sequence of the L-CPT I gene, DNase I hypersensitive sites (Hss) were mapped. Because rat hepatic CPT I mRNA is strongly induced during the first day of extrauterine life (5), we compared the chromatin structure of the L-CPT I gene from 1-day-old suckling rats to that of fetuses in which the L-CPT I gene was weakly expressed. Neonatal spleen, which did not express

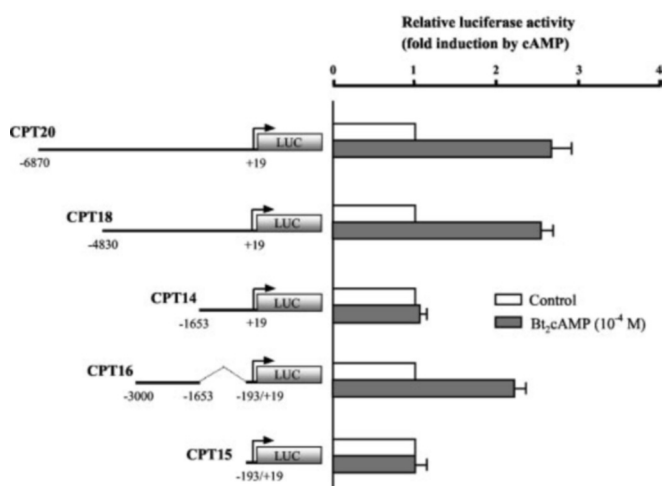


FIG. 2. Identification of a cAMP-responsive unit in the L-CPT I promoter gene. Primary hepatocytes from 12-day-old suckling rats were done as described under "Experimental Procedures." After 4 h of plating, cells were transfected for 7 h with 2.5 μ g of each plasmid and then cultured in the presence or absence of Bt₂cAMP (10⁻⁴ M) for 24 h. Successive deletions of the L-CPT I promoter from -6870 bp to -1653 bp of the transcription start site were tested. After 24 h of culture, cells were harvested and assayed for protein concentrations and luciferase activity. Luciferase assays (relative light units) were normalized using pGL3-enhancer vector activity standard. Each transfection was conducted in triplicate and repeated three to six times. Results are means \pm S.E. of four different experiments.

L-CPT I, was included as a negative control. Two Hss (Hss1 and Hss2) were mapped in a 10-kb *Eco*RI fragment (Fig. 3A). Hss1 and Hss2 located at -2.5 kb and -3 kb from the transcription start site, respectively, were observed in the liver of both fetuses and suckling rats, but not in spleen (Fig. 3A). The presence and the precise localization of these two distal sites were confirmed by using the probe S2 (Fig. 3B). Significantly, both Hss1 and Hss2 were stronger in neonatal liver than in the corresponding fetal tissue. Thus, the neonatal induction of L-CPT I is correlated with changes in the DNase I accessibility of the promoter. To identify putative cAMP-response elements in this region a computer-based search for known DNA-binding elements was conducted on the L-CPT I promoter (-3000 to -1654). One CRE-like motif located around -2900 bp was identified.

The CRU of the L-CPT I Gene Is Composed by two DNA Elements, a CRE and a DR1 Motif—To further characterize the sequences involved in the cAMP induction of the L-CPT I gene transcription, a 200-bp sequence (-2967 to -2773) including the CRE-like motif linked to the thymidine kinase promoter was transfected into primary rat hepatocytes. A 3.7-fold increased in reporter gene activity was observed in the presence of cAMP (Fig. 4). Similar results were observed in the presence of glucagon, suggesting that the pancreatic hormone was indeed stimulating L-CPT I gene expression through the 200-bp DNA sequence containing the CRE motif (Fig. 4).

To test the functional role of the CRE-like motif in the cAMP response of the L-CPT I gene, a construct containing two copies of the CRE sequence (CRE2x) was transfected. No specific stimulation by cAMP was observed, whereas the well characterized CRE sequence of the somatostatin promoter (19) was induced by 3.3-fold in the same culture conditions (Fig. 4). Thus, the CRE motif is insufficient to mediate the cAMP response of the L-CPT I gene. We reasoned that this induction probably requires cooperation between several DNA sequences contained within the 200-bp fragment (-2967 to -2773).

Putative binding sequences for several transcription factors exist within the 200-bp fragment (-2967 to -2773) of the L-CPT I promoter (Fig. 5A). Thus, in addition to the CRE motif,

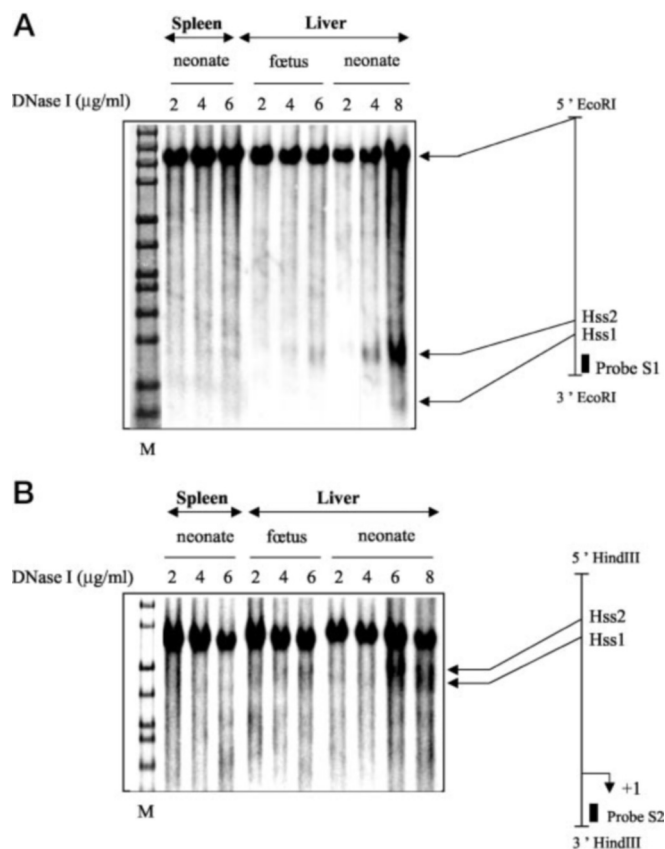


FIG. 3. Determination of DNase I hypersensitive sites in the rat L-CPT I gene promoter. Nuclei were prepared from livers of 21-day-old fetuses and from livers and spleens of 1-day-old newborn rats. They were digested with DNase I at the final concentration indicated above each line. DNase I hypersensitive sites are indicated with arrows at the right of the figure. A, autoradiogram was done with the probe S1, which corresponding to the 744-bp *Eco*RI-*Sph*I fragment localized at the -1653 bp from the transcription start site of the L-CPT I gene. B, autoradiogram was done with the probe S2 corresponding to an 855-bp *Hind*III-*Bam*HI fragment located in the first intron of the L-CPT I gene. M represents end-labeled size marker of 22.0-19.3, 13.3, 10.1, 7.7, 6.2, 4.2, 3.5, 2.7, 1.9, 1.5, 0.9, and 0.6 kb.

DR1, C/EBP, and two AP1 motifs (named AP1(a) and (b)) are also present in this region. Successive deletions of each potential transcription factor binding motif were performed to define the regulatory elements involved in the cAMP response of the L-CPT I gene. No modification of reporter activity was observed after cAMP stimulation when the C/EBP and/or the AP1(a) boxes were deleted relative to the cAMP activation of the entire 200-bp fragment (Fig. 5A). However, the deletion of the DR1 motif decreased the reporter gene activity by about 70% (Fig. 5A). Thus, the fragment (-2938 to -2831) containing the CRE, AP1(b), and DR1 motifs is sufficient to reproduce the cAMP response of the 200-bp fragment (-2967 to -2773) (Fig. 5A). Substitution of four bases in the AP1(b) box had no effect on reporter activity (Fig. 5A). Moreover, the complete deletion of the DNA sequence located between CRE and DR1 motifs, which contained the AP1(b) box, led to only a weak decrease (about 30%) in CAT activity compared with the cAMP response of the fragment (-2938 to -2831) (Fig. 5A). Deletion or mutation of either of these two motifs completely disrupted the cAMP-elicited induction of reporter activity (Fig. 5B). Thus, both the CRE and DR1 motifs are important for the induction of the L-CPT I gene by cAMP. Taken together, these data strongly suggest that the functional CRU of the L-CPT I gene is composed of a CRE and a DR1 that are both necessary and sufficient for induction by cAMP.

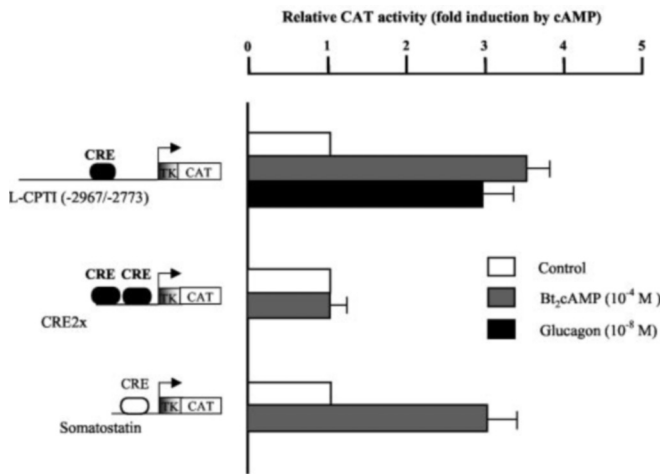


FIG. 4. Effects of Bt₂cAMP and glucagon on the cAMP-responsive region of the L-CPT I gene. Primary hepatocytes were transfected with 2.5 μ g of the heterologous promoter-reporter constructs L-CPT I (-2967 to -2773) and CRE2x or the CRE sequence of the somatostatin promoter, which was used as a positive cAMP target construct. Hepatocytes were cultured with or without Bt₂cAMP (10⁻⁴ M). After 48 h of culture, cells were harvested and assayed for protein and CAT assays. The pSV2CAT vector was used as control to normalize CAT activity. Results are means \pm S.E. of three different experiments.

Identification of the Transcription Factors Involved in the Induction of L-CPT I by cAMP—Cotransfection experiments were performed with expression vectors encoding proteins potentially involved in the cAMP transduction signal pathway (12). We examined the ability of the catalytic subunit of PKA (19) or a dominant positive form of the transcription factor CREB (VP16-CREB) to mimic cAMP-mediated induction of the L-CPT I gene fragment (-2938 to -2831). Cotransfection of these factors had no effect on the empty vector (Fig. 6). In contrast, the 4-fold induction of the L-CPT I (-2938 to -2831) construct by cAMP was mimicked by cotransfection with either the catalytic subunit PKA vector or the CREB dominant positive expression vector (Fig. 6). Moreover, the response of the L-CPT I promoter to cAMP was abolished by cotransfection of a dominant negative mutant of CREB. Thus, CREB can activate the L-CPT I promoter.

We next considered possible factors that could affect transcription via the DR1 element. We have shown previously that the DR1 element of the L-CPT I CRU binds PPAR α (11). However, induction of the L-CPT I gene by cAMP is not abolished in primary culture of hepatocytes from PPAR α KO mice.² So, it seems that PPAR α is not directly involved in the cAMP activation of the L-CPT I gene. Significantly, a recent study showed that stimulation of PEPCK gene expression occurs via HNF4 α through two DR1-like motifs (16). We therefore considered the possibility that HNF4 α is the DR1 element-binding protein. Consistent with this hypothesis, transfection of a dominant negative mutant of HNF4 α abolished the response of the L-CPT I promoter to cAMP (Fig. 6).

HNF4 α and CREB DNA-binding Activity to the L-CPT I CRU—To determine whether CREB and HNF4 α directly interact with the CRU of the L-CPT I gene, gel shift assays were performed. We first tested the ability of CREB to bind the CRE motif of the L-CPT I gene. In the presence of recombinant CREB protein (r-CREB) a major complex was detected. Formation of this complex was inhibited by an excess of unlabeled CRE probe (Fig. 7). By using rat liver nuclear extracts, a supershift was obtained in the presence of the CREB antibody demonstrating that the protein CREB contained in these nu-

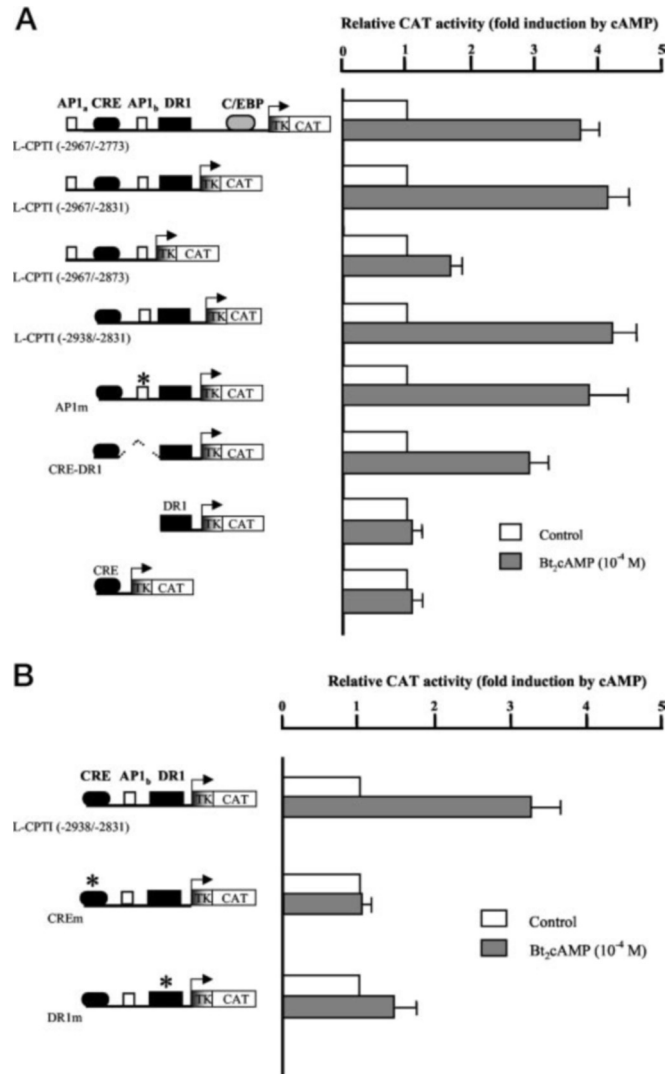


FIG. 5. Identification of the elements involved in the cAMP-responsive unit. Hepatocytes were transfected and cultured as described in Fig. 4. A, successive deletions in the 5' and 3' end of the 200-bp DNA fragment (-2967 to -2773) driven by the TKCAT plasmid and specific mutation in the short fragment of the 107-bp of AP1 sequence (AP1m), deletion of DNA sequences between CRE and DR1 sequences (CRE/DR1), and CRE and DR1 sequence driven by the TKCAT plasmid. Results are means \pm S.E. from three to five different experiments. B, site-directed mutagenesis of the CRE and the DR1 motif was conducted as described under "Experimental Procedures." These vectors were transfected into primary cultured hepatocytes and assessed for cAMP responsiveness as described in Fig. 4. Results are means \pm S.E. of three different experiments.

clear extracts is able to bind the L-CPT I CRE element (Fig. 7). The well characterized somatostatin CRE probe showed a similar supershift pattern with CREB antibody in the presence of the same liver nuclear extracts (Fig. 7) (19).

We next tested the ability of HNF4 α to bind the DR1 motif. A labeled DNA fragment spanning the DR1 motif of the L-CPT I promoter was incubated with recombinant proteins or *in vitro* translated HNF4 α protein (Fig. 8A). A major complex was detected with both recombinant proteins and *in vitro* translated HNF4 α (Fig. 8A, lanes 2–4). Addition of anti-HNF4 α antibody resulted in a supershifted complex (Fig. 8A, lane 5). Formation of the complex was also inhibited with an excess of unlabeled DR1 probe (Fig. 8A, lanes 6–8). Similar data were obtained using a CRE-DR1 probe (data not shown), while no specific complex was detected with a mutated DR1 probe in the presence of r-HNF4 α (data not shown). These results suggest

² J. P. Pégrier, personal communication.

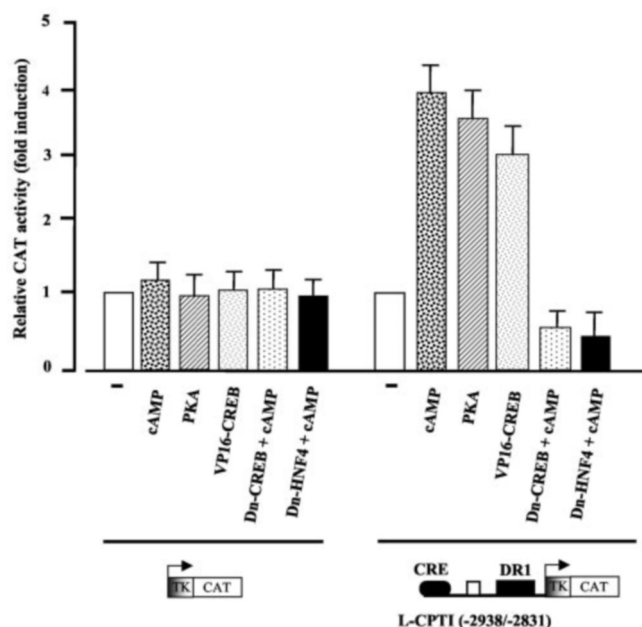


FIG. 6. Transcription factors involved in the induction of L-CPT I gene by cAMP. Hepatocytes were cultured and cotransfected with 2.5 μ g of the L-CPT I (–2938 to –2831) or the empty plasmid and 1 μ g of several expression vectors as described under “Experimental Procedures”; cotransfection with 1 μ g of PKA (Ca), 1 μ g of VP16-CREB, and 1 μ g of dominant negative mutants; and Dn-CREB or Dn-HNF4 in the presence of Bt2cAMP (10^{-4} M). After 48 h of culture, cells were harvested and assayed for protein and CAT assays. Results are means \pm S.E. of three different experiments.

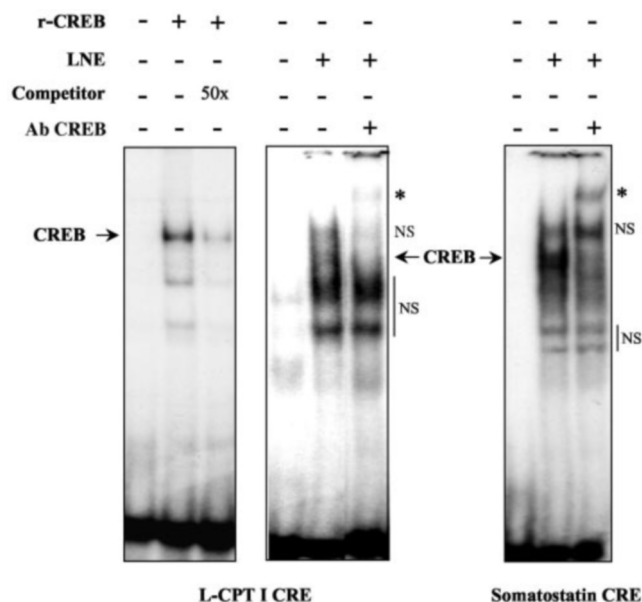


FIG. 7. Binding of nuclear proteins to the L-CPT I CRE motif. Gel shift assays were performed with a CRE 32 P-labeled double strand oligonucleotide from the L-CPT I and somatostatin genes. The labeled probe containing the L-CPT I CRE was incubated with 50 ng of recombinant CREB (r-CREB) and nuclear proteins isolated from rat liver as described under “Experimental Procedures.” Incubation is also conducted in the presence of L-CPT I CRE unlabeled competitor oligomer added from 50-fold molar excess as indicated and CREB antibody. The arrow denotes the position of the complex containing CREB, and the supershift band is indicated by the asterisk. The result is representative to three separate experiments. NS, nonspecific.

that HNF4 α binds the DR1 motif of the L-CPT I promoter *in vitro* with high affinity. EMSA were performed with crude nuclear extracts from cultured hepatocytes to determine

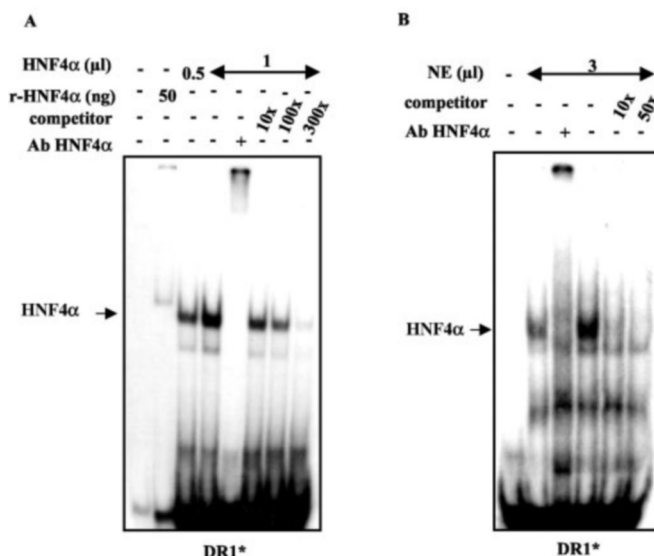


FIG. 8. Binding of HNF4 α to the L-CPT I DR1 motif. Gel shift assays were performed with a 32 P-labeled double strand oligonucleotide as described under “Experimental Procedures.” A, the labeled probe containing the L-CPT I DR1 was incubated with 0.5 or 1 μ l of *in vitro* translated HNF4 α or 50 ng of recombinant HNF4 α (r-HNF4 α). Incubation is also conducted in the presence of HNF4 α antibody for the gel supershift analysis or unlabeled competitor oligomer added from 10- to 300-fold molar excess as indicated. B, gel shift assays were performed by using the same probe incubated in the presence of nuclear proteins isolated from rat hepatocytes. The arrow denotes the position of the complex containing HNF4 α . The result is representative to three separate experiments.

whether the DR1 of the L-CPT I gene was able to bind endogenous HNF4 α . EMSA with the DR1 probe using nuclear extracts revealed a complex of low mobility (Fig. 8B, see arrow) that migrated similarly to that observed with *in vitro* translated HNF4 α (Fig. 8A). This complex could be supershifted using an anti-HNF4 α antibody (Fig. 8B, lane 3). Addition of an excess of unlabeled DR1 probe markedly diminished the intensity of the low mobility complex indicating that this interaction was specific (Fig. 8B). Thus, HNF4 α binds to the DR1 element of the L-CPT I promoter both *in vitro* and *in vivo*.

Expression of the L-CPT I Gene Is Diminished in Liver-specific H4LivKO—It is becoming increasingly clear that the extrapolation of data from transfection studies to the *in vivo* situation must be made with caution. For example, both ApoA-I and ApoE are positively regulated by HNF4 α in transient transfection assays, but expression of these genes is essentially unaffected by disruption of the HNF4 α gene in the adult mouse (31). To determine if HNF4 α is involved in L-CPT I gene expression *in vivo*, we measured steady-state mRNA levels in the livers of H4LivKO mice (31). In H4LivKO mice, disruption of a loxP-targeted allele of HNF4 α is driven by an albumin-Cre transgene, resulting in efficient, liver-restricted depletion of HNF4 α protein. Consistent with our transfection data, L-CPT I expression was significantly lower in the liver of H4LivKO mice compared with controls (Fig. 9).

The Coactivator PGC-1 Potentiates the Induction of the L-CPT I Gene by cAMP—The involvement of PGC-1 has been shown in the regulation of gluconeogenic genes by cAMP and glucocorticoids (16). It has been demonstrated that PGC-1 is transcriptionally activated (via CREB) after 6–8 h of treatment with cAMP in hepatocytes (16). To determine if this induction precedes the up-regulation of L-CPT I in our system, we tested the effects of cAMP (10^{-4} M) or glucagon (10^{-8} M) on expression of endogenous PGC-1 in cultured hepatocytes (Fig. 10A). An increase of PGC-1 mRNA concentration was detected

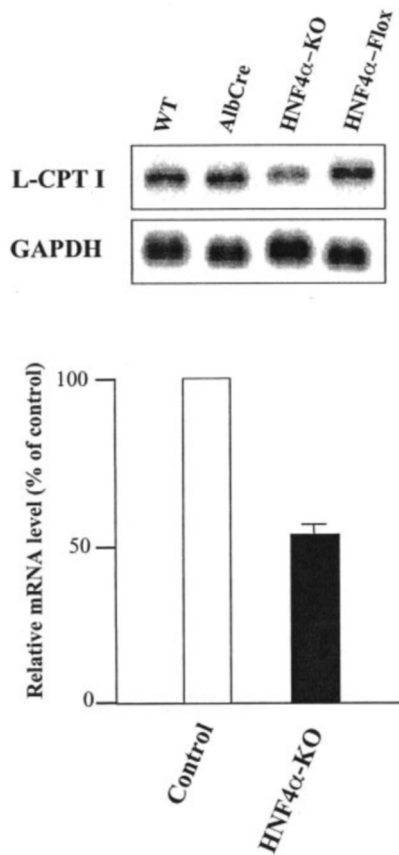


FIG. 9. Northern blot analysis of the L-CPT I gene expression in the liver of HNF4^{-/-} mice. Poly(A)⁺ RNAs were isolated from livers of 45-day-old H4LivKO and control mice (44). Northern blots were performed using standard procedures and the filter hybridized with a cDNA probe specific for L-CPT I. As a loading control, the same blot was stripped and rehybridized with a probe specific for glyceraldehyde-3-phosphate dehydrogenase. Genotypes of mice are as follows: WT (control), HNF4 w/w; AlbCre ^{-/-}; AlbCre, HNF4 w/w, AlbCre ^{+/+}; HNF4α-KO, HNF4 fl/fl, AlbCre ^{+/+}; HNF4α-Flox, HNF4 fl/fl, AlbCre ^{-/-}.

after 4 h in the presence of cAMP or glucagon with a maximal induction observed after 24 h of culture (Fig. 10A). By comparison, induction of PEPCK mRNA was detectable within the first hour of cAMP incubation, whereas L-CPT I mRNA accumulation occurred only after 48 h (Fig. 10A). Thus, the induction of PGC-1 mRNA precedes that of L-CPT I but not PEPCK. This observation is consistent with an essential role for PGC-1 in the control of L-CPT I expression by cAMP.

To further investigate the role of PGC-1 in the induction of the L-CPT I gene by cAMP, dose-response experiments were done. PGC-1 was cotransfected in a dose-dependent manner with the L-CPT I (-2938 to -2831) construct in the presence or in the absence of a regular cAMP concentration. PGC-1 markedly increased the level of CAT activity with the highest PGC-1 concentration tested (1000 ng/well) in the absence of cAMP. Under these conditions, the addition of cAMP had no effect on PGC-1-mediated induction suggesting a convergent molecular pathway between cAMP and PGC-1 in the transcriptional induction of the L-CPT I gene. At low concentrations of PGC-1, no synergic effects were observed between cAMP and the coactivator (Fig. 10B). These data suggest that PGC-1 mediates the cAMP response of the L-CPT I gene.

To identify the regulatory elements mediating the effect of PGC-1, mutant constructs of the L-CPT I CRU (-2938 to -2831) were tested. Mutation of the DR1 element strongly diminished the reporter activity, whereas the double mutant

construct (DR1m-CREm) completely abolished CAT activity (Fig. 10C). A similar result was obtained when PGC-1 was cotransfected with the dominant negative form of HNF4α (Fig. 10C). These data indicate that the activation of L-CPT I gene by PGC-1 occurs through the liver-enriched transcription factor HNF4α that binds to the DR1 motif.

DISCUSSION

Hormones and neurotransmitters change patterns of gene expression in target cells by activating protein kinases that phosphorylate and modify the activity of different transcription factors (32). CREB, the first transcription factor whose activity was shown to be regulated by phosphorylation, mediates the transcriptional effect of cAMP by binding the CRE element in the upstream region of a number of genes (33). This transcription factor is phosphorylated by PKA after an increase of intracellular cAMP concentration. More recently, it was demonstrated that CREB is an *in vivo* substrate for a variety of other kinases like the calmodulin kinase, the mitogen-activated protein kinase/p90rsk, the protein kinase C and the protein kinase B (PKB/Akt) (34). This suggests that CREB could activate transcription of genes in response to various other extracellular signals such as Ca²⁺ or growth factors (35–37). Thus, the molecular mechanisms mediating the cAMP signal and its specificity are more complex than previously thought.

In the present study, we have examined the regulation of L-CPT I gene expression by cAMP in hepatocytes. Our results show that two cis-acting elements, a DR1 motif and a CRE-like element (TGACG), are required for the full stimulatory effect of cAMP on L-CPT I expression. This type of “multi-responsive element” structure, termed CRU, is not a surprising observation. Indeed, it is now generally accepted that transcriptional regulation of genes by cAMP seldom occurs through a unique CRE motif as described for the somatostatin gene (38). For example, the full cAMP induction of glucose-6-phosphatase gene expression involves two CRE elements in HepG2 cell lines (39, 40), while in kidney cell lines, the same gene is regulated by cAMP through these two CRE sequences and an HNF1 motif (41). Furthermore, the CRU of the TAT gene, which like L-CPT I, is induced by glucagon and repressed by insulin, is composed of a CRE and a DR1 motif (14). The L-CPT I gene contains four consensus CRE sites, from CRE1 to CRE4 (6). Interestingly, the classical, symmetric CRE1 (TGACGTAA), which differs from the well characterized CRE sequence of the rat somatostatin gene (TGACGTCA) by only one nucleotide, is not sufficient for the response to cAMP. These data strongly suggest that the presence of CRE consensus sequences within the promoter of genes is not sufficient to fully explain cAMP gene activation. Our study shows that the functionality of a CRE sequence is more dependent on its promoter context (proximity to other cis-acting element(s), localization in an open chromatin structure) than on its homology to the CRE consensus sequence. Furthermore, the precise DNA structure of the L-CPT I CRU is required for the full stimulatory effect of L-CPT I gene expression by cAMP as deletions of the DNA sequence located between the CRE and the DR1 motifs substantially reduced the cAMP response. Therefore, accurate distance between the CRE and DR1 motifs is required for their cooperative actions. Similar data have been obtained for the cAMP-inducible enhancer of the urokinase-type plasminogen activator gene (42).

The DNA structure of the L-CPT I CRU suggested a potential role for proteins involved in the cAMP signaling pathway, (*i.e.* PKA and CREB) in transcriptional activation through the CRE-like sequence. Experimental evidences for the involvement of CREB in L-CPT I gene activation by cAMP were as follows: (i) in cotransfection experiments, PKA and CREB mimic the cAMP induction of the constructs containing the

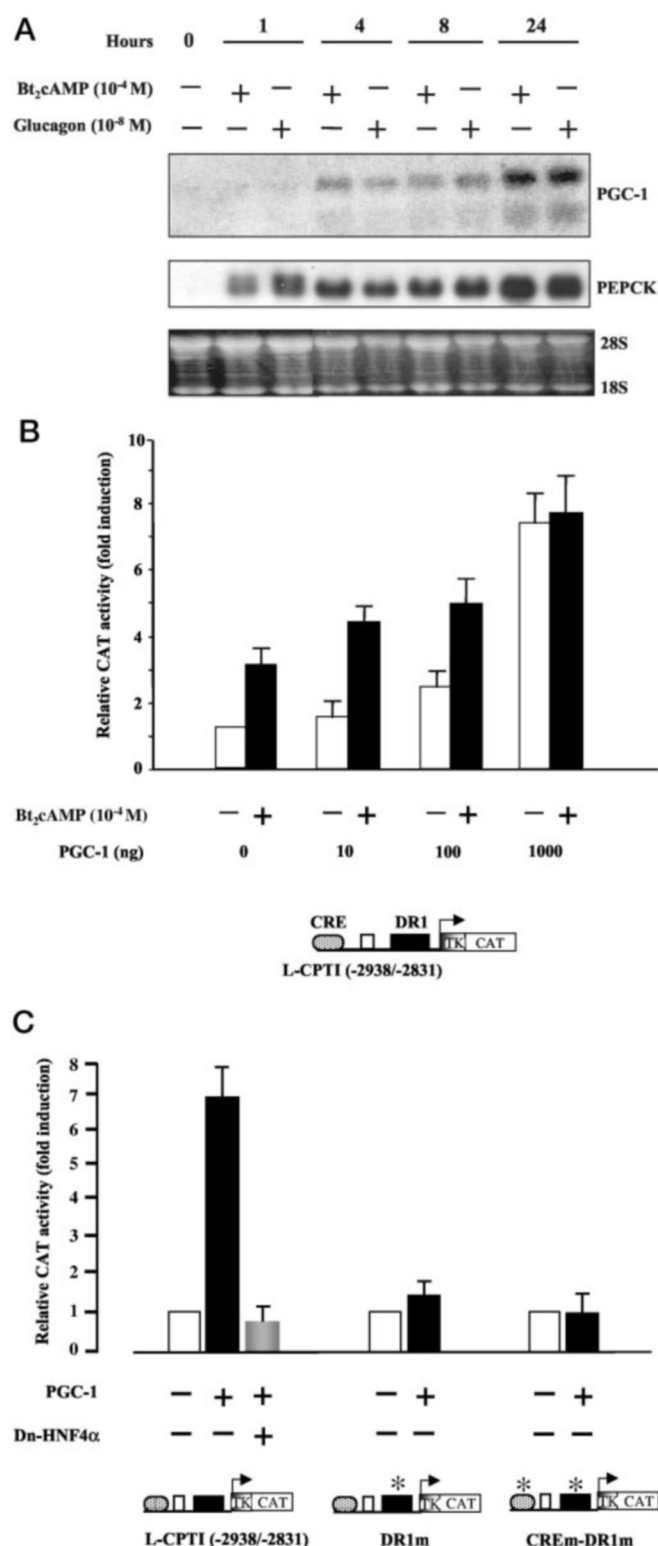


FIG. 10. Role of PGC-1 in the induction of L-CPT I gene by cAMP. Hepatocytes were cultured as described previously under "Experimental Procedures." **A**, hepatocytes were cultured in the presence of glucagon (10⁻⁸ M), Bt₂cAMP (10⁻⁴ M) or in the absence of the two. At the indicated time, cells were scrapped, total RNA was extracted, and the level of PGC-1 mRNA was measured by Northern blot. Experiments were performed twice with identical results. **B**, L-CPT I (-2938 to -2831) wild-type vector was cotransfected with an increasing rate of PGC-1 expression vector in primary hepatocytes. After 48 h of culture in the presence or absence of Bt₂cAMP (10⁻⁴ M), cells were harvested and assayed for protein and CAT assays. **C**, cells were cotransfected with 2.5 μ g of the L-CPT I (-2938 to -2831) wild-type or DR1m or CREm-DR1m constructs in the presence of 1 μ g of PGC-1, HNF4 α dominant negative

CRU element, (ii) cotransfections of a CREB dominant negative abolished the specific cAMP induction of L-CPT I CRU, and (iii) in gel shift experiments, a recombinant CREB protein binds the asymmetric CRE sequence of the L-CPT I CRU. However, this CRE element cannot alone elicit the full cAMP response of the L-CPT I gene as a multimerized CRE construct was not sufficient for the cAMP response.

Transfection experiments clearly demonstrated a major role for the DR1 motif in cAMP-mediated induction of the L-CPT I gene as site-directed mutagenesis of the DR1 motif strongly reduced the cAMP induction of the L-CPT I CRU. Previous studies have demonstrated that the nuclear receptor HNF4 α was involved in the cAMP responsiveness of the TAT gene through the DR1 motif (14). Based on these data, the potential implication of HNF4 α in the cAMP induction was studied. Using gel shift experiments, we show that this liver-enriched transcription factor can bind the DR1 sequence of the L-CPT I gene with high affinity. In addition, cotransfection of an HNF4 α dominant negative in primary cultures of hepatocytes completely abolished the cAMP responsiveness of the L-CPT I gene. Taken together, these data suggest that the transcriptional regulation by cAMP gene involves HNF4 α in addition to the classical CREB family members.

The liver-specific disruption of the HNF4 α gene has recently been achieved using the Cre-loxP system (31). This study provides the first direct *in vivo* evidence that HNF4 α is required for the constitutive expression of several key hepatic genes involved in both lipid transport and metabolism including Apo-AII, Apo-CII, Apo-CIII, and L-FABP genes (31). HNF4 α probably exerts its effect via recruitment of coactivators, allowing specific induction of hepatic genes by remodeling of chromatin (43). This potential role for HNF4 α is in close agreement with our data. The liver-specific open chromatin region around the CRU corresponding of DNase I hypersensitive site Hss2 could be a direct or indirect consequence of the HNF4 α activity. This transcription factor is probably required for the constitutive expression of the L-CPT I gene because HNF4 α was found to bind the DR1 motif of the L-CPT I gene in nuclear extracts of hepatocytes cultured in the absence of cAMP. This hypothesis was confirmed by the fact that the basal activity of the L-CPT I gene expression is significantly reduced in liver HNF4 α knock-out mice. However, these molecular mechanisms cannot explain the late phase of induction of the L-CPT I gene by cAMP. Usually, transcription of cellular genes by cAMP increases after 30 min of stimulation coinciding with the time required for catalytic subunit levels of the PKA to become maximal in the nucleus (12). Moreover, it was well established that pancreatic hormones exert only a long term effect on L-CPT I activity. Hence, neither insulin nor glucagon are able to exert short term control on L-CPT I activity either *in vivo* (44, 45) or *in vitro* (46). Thus, it cannot be excluded that other factors involved in the L-CPT I gene regulation by cAMP remain to be identified.

Evidence has emerged that nuclear receptors regulate transcription via interactions with coactivators like CBP/p300, SRC1, PGC-1, or corepressors like SMRT and N-CoR (47). Recently, the nuclear receptor coactivator PGC-1 was shown to transcriptionally regulate the response of key gluconeogenic genes (PEPCK, glucose-6-phosphatase, fructose-1,6-bisphosphatase) to cAMP and/or glucocorticoids (16), as well as the genes encoding enzymes of mitochondrial fatty acid oxidation (medium-chain acyl-CoA dehydrogenase, and long-chain acyl-

mutant expression vectors, or the empty TKCAT vector. After 48 h of culture, cells were harvested and assayed for protein and CAT assays. For **B** and **C**, results are the means \pm S.E. of four different experiments.

CoA dehydrogenase) in 3T3-L1 preadipocytes (16). We therefore tested the possibility that PGC-1 could serve as a coactivator for the transcriptional control of the L-CPT I gene by cAMP. Our results indicate that the endogenous PGC-1 gene is inducible by both glucagon and cAMP in isolated hepatocytes. It has been previously shown in HepG2 cells that the promoter of the PGC-1 gene contains a functional CRE element (16). Interestingly, the cAMP-induced accumulation of PGC-1-mRNA precedes that of L-CPT I, suggesting that the primary induction of the PGC-1 gene by cAMP could be important for L-CPT I gene activation. To further understand the molecular mechanisms involved in the PGC-1-induced transactivation of L-CPT I, transient transfections in primary cultures of hepatocytes were performed. As it has been described for PEPCK (16) and GLUT4 genes (48), high levels of PGC-1 activate the L-CPT I gene promoter in the absence of exogenous hormones. Mutation in the DR1 motif or cotransfection with the HNF4 α dominant negative mutant abolished the PGC-1 effect. These data suggest that: (i) the PGC-1-mediated transactivation of the L-CPT I gene is specific to the CRU, and (ii) the DR1 motif and the adequate HNF4 α binding are necessary for the maximal PGC-1 activation. Interestingly, a recent study has suggested that PGC-1 activates the PEPCK gene promoter and acts in interaction with HNF4 α through two DR1-like motifs called gAF1 and gAF3 (16). These two transcription factor sites have been shown to bind HNF4 α , COUP-TF, PPAR γ , or PPAR α , respectively. In previous experiments, we have also shown that the DR1 element of the L-CPT I CRU could bind PPAR α (11). However, it seems that PPAR α is not directly involved in the cAMP activation of the L-CPT I gene as, in primary culture of hepatocytes from PPAR α -/- mice, L-CPT I gene induction by cAMP is not abolished.² The fact that PGC-1 overexpression is sufficient to induce specific genes, including L-CPT I, suggests that DNA-binding proteins are already present and that the intracellular concentration of the coactivator is limiting (49). Therefore, as it has been suggested by Puigserver *et al.* (50), PGC-1 transactivation of these target genes could be summarized in a molecular model: (i) PGC-1 docks to the transcription factors already bound to their appropriate DNA sequence such as HNF4 α and CREB for the L-CPT I gene and (ii) PGC-1 after its docking undergoes a conformational change that is permissive for cofactor binding (50). At this state PGC-1 has obtained a maximal capacity to assemble a complex involving other coactivators such as CBP/p300 and thus activate transcription. The gradual assembly of these protein complexes would allow an accurate tissue and temporal regulation of target genes. Our study explored the first step of the molecular mechanisms involved in the cAMP activation of the L-CPT I gene. We show for the first time that PGC-1 is directly involved in the regulation of a liver-specific gene of the lipid metabolism by cAMP. The regulation of this gene, in response to glucagon, requires the formation of a multi-protein complex including the coactivator PGC-1, CREB, and HNF4 α . The formation of this multiprotein complex involved in the transcriptional regulation of target genes is technically difficult to observe in a cellular context. However, physical interaction between PGC-1 and HNF4 α has been previously shown *in vitro* (16). Interaction between PGC-1 and HNF4 α occurs between the LXXLL motif in the N terminus of PGC-1 and the AF-2 domain of HNF4 α (16). Moreover, PGC-1 also physically interacts with CBP *in vitro* (50). Together these data suggest that a protein complex containing the coactivator PGC-1, HNF4 α , and CREB could effectively form in hepatic cells.

Uncontrolled mitochondrial fatty acid oxidation and the resulting ketogenesis are important risk factors in human diseases such as type I diabetes. Understanding the mechanisms controlling fatty acid oxidation therefore has potential impli-

cations for the understanding and treatment of these diseases. We demonstrate, for the first time, a prominent role for PGC-1 in the control of mitochondrial fatty acid oxidation in the liver. Thus, in addition to the key role that PGC-1 plays in glucose homeostasis, it may also be critical for lipid homeostasis. Taken together these observations suggest that PGC-1 acts to coordinate the process of metabolic adaptation in the liver.

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