Carnitine palmitoyltransferase I (CPT-I) catalyzes the rate-controlling step in the pathway of mitochondrial fatty acid oxidation. Thyroid hormone will stimulate the expression of the liver isoform of CPT-I (CPT-I\(\alpha\)). This induction of CPT-I\(\alpha\) gene expression requires the thyroid hormone response element in the promoter and sequences within the first intron. The peroxisomal proliferator-activated receptor-\(\gamma\) coactivator-1\(\alpha\) (PGC-1\(\alpha\)) is a coactivator that promotes mitochondrial biogenesis, mitochondrial fatty acid oxidation, and hepatic gluconeogenesis. In addition, PGC-1\(\alpha\) will stimulate the expression of CPT-I\(\alpha\) in primary rat hepatocytes. Here we report that thyroid hormone will increase PGC-1\(\alpha\) mRNA and protein levels in rat hepatocytes. In addition, overexpression of PGC-1\(\alpha\) will enhance the thyroid hormone induction of CPT-I\(\alpha\) indicating that PGC-1\(\alpha\) is a coactivator for thyroid hormone. By using chromatin immunoprecipitation assays, we show that PGC-1\(\alpha\) is associated with both the thyroid hormone response element in the CPT-I\(\alpha\) gene promoter and the first intron of the CPT-I\(\alpha\) gene. Our data demonstrate that PGC-1\(\alpha\) participates in the stimulation of CPT-I\(\alpha\) gene expression by thyroid hormone and suggest that PGC-1\(\alpha\) is a coactivator for thyroid hormone.

The rate-controlling step in the pathway of mitochondrial fatty acid oxidation is catalyzed by carnitine palmitoyltransferase I (CPT-I)\(^1\). CPT-I, which is located on the outer mitochondrial membrane, catalyzes the transfer of long chain fatty acids from acyl-CoA to carnitine to form acyl carnitine. Acyl carnitine is translocated across the mitochondrial membrane by carnitine acyl-carnitine translocase (1). CPT-II on the inner mitochondrial membrane transfers the acyl group back to CoA. The two main isoforms of CPT-I are the liver isoform (CPT-I\(\alpha\)) and the muscle isoform (CPT-I\(\beta\)) (1, 2). CPT-I\(\alpha\) is expressed in all tissues except skeletal muscle and adipose tissue, whereas CPT-I\(\beta\) is present in several tissues including the heart, skeletal muscle, brain, and fat (3–5). Heart and brain express both CPT-I isoforms (4, 5).

The oxidation of long chain fatty acids in the liver is elevated in hyperthyroidism, fasting, and streptozotocin-induced diabetes (6, 7). We have observed that the expression of the rat CPT-I\(\alpha\) gene and CPT-I\(\alpha\) activity are increased in these states (7, 8). In the liver, there is a 40-fold increase in CPT-I\(\alpha\) mRNA abundance between hypothyroid and hyperthyroid animals (7). Our laboratory has cloned the promoter for the rat CPT-I\(\alpha\) gene and identified a thyroid hormone response element (TRE) in the CPT-I\(\alpha\) promoter at nucleotides –2938/–2923 (9–11). We have shown that the CPT-I\(\alpha\)-TRE binds the thyroid hormone receptor (TR) as a heterodimer with the retinoid X receptor (10). There are several unusual features of the induction of CPT-I\(\alpha\) by T3. First, whereas CPT-I\(\alpha\) is strongly induced by T3 in the liver, CPT-I\(\alpha\) is not induced by T3 in the heart (5, 12). In addition, sequences within the first intron contribute to the high expression of CPT-I\(\alpha\) in the liver and are necessary for the full induction of the CPT-I\(\alpha\) gene by T3 (10, 12).

It is now clear that coactivators have a crucial role in the regulation of gene expression by T3 (13). Several families of coactivators have been shown to interact with the liganded TR including steroid receptor coactivators (SRC), CREB-binding proteins (CBP/p300), and the thyroid hormone activator proteins (TRAPs) (14). These coactivators will stimulate gene expression, acetylate histones, and mediate interactions between transcription factors (13). The peroxisomal proliferator-activated receptor-\(\gamma\) coactivator (PGC-1) is a coactivator that was initially identified as a protein that interacted with the peroxisomal proliferator-activated receptor-\(\gamma\) (15). Several isoforms of PGC-1 have been cloned including PGC-1\(\alpha\), PGC-1\(\beta\), and PGC-related coactivator (16–18). PGC-1\(\alpha\) is highly expressed in tissues with high metabolic rates including heart, muscle, and brown adipose tissue (15). Overexpression of PGC-1\(\alpha\) in heart and muscle promotes mitochondrial biogenesis and increased energy expenditure (19). In the heart, many genes involved in fatty acid oxidation such as medium chain acyl-CoA dehydrogenase and CPT-I\(\beta\) are stimulated by PGC-1\(\alpha\) (19, 20).

The unique properties of PGC-1\(\alpha\) as a coactivator are that it has a limited tissue specific pattern of expression and that the transcription of PGC-1\(\alpha\) is induced by environmental changes such as cold and hormones (15). In the liver, PGC-1\(\alpha\) abundance is increased in fasting and diabetes (21). PGC-1\(\alpha\) will promote hepatic gluconeogenesis through its stimulation of the genes for phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (Glc-6-P) (21–23). PGC-1\(\alpha\) can interact
The following primers were used for real time PCR to quantitate mRNA abundance

| Gene     | Primer 1                     | Primer 2                     |
|----------|------------------------------|------------------------------|
| CPT-I    | AGAATGCGACGGCTTCTAGGC        | AAGCGTCCTGAATTCCTCACAGC      |
| PGC-1α   | ACGCAGGAGTCTCATGTTCA        | GAGAGCAATGGCAGGGTTTGTTC      |
| CPT-I    | CCGTACAAAGATGGCATGTCATC     | AAACAATCAGTGCACCGACAGT      |
| CPT-Iα   | TCACACCACACACACAGAGT        | GGCGCAGAAGATTTCAAGAT       |
| CPT-Iα   | GAGCGGTACCACTACGAAAAAGAA    | GGCTTGTCACTACTCCTCCCC      |

The following primers were used for the ChIP assay to demonstrate interactions of PGC-1α with the CPT-Iα gene

| Primer Set | Forward Primer | Reverse Primer |
|------------|----------------|----------------|
| Rat CPT-Iα TRE primers | GAACGACGGGTGATCATTTCAACAG | CAGGACATGTGTTTTTCTACAG |
| Rat CPT-Iα intron 1 primers | AAAGCAGTCTCAAGTTCGCGGT | GAGAGCTTCGAGGATTTGTTG |
| Rat CPT-Iα upstream promoter primers | CTCTGGTGTCCTGTAACCTGTGG | GAAGGAGTCTGTTACCACTGTCAG |
| Rat PEPCK primers | AACTCGCTGGTTGGCAGCCTT | CATCAGCAACAGTCAGGTC |

MATERIALS AND METHODS

Transient Transfection of Luciferase Vectors—CPT-Iα-luciferase constructs (CPT-Iα-luc) were transiently transfected into HepG2 cells by the calcium phosphate method (10). Transfections included 2 μg of CPT-Iα-luciferase along with RSV-thyroid hormone receptor β (RSV-TRβ) and TK-Renilla vectors. Cells were transfected in Dulbecco’s modified Eagle’s medium containing 5% calf serum, 5% fetal calf serum and incubated overnight at 37 °C. Following two washes with phosphate-buffered saline, the medium was replaced by Dulbecco’s modified Eagle’s medium containing no serum. Cells were treated with 100 nM T3 for 24 h. After T3 treatment, cells were lysed in Passive Lysis Buffer (Promega). Both luciferase and Renilla activity were measured. Protein content in each lysate was determined by Bio-Rad Protein assay (Bio-Rad). Luciferase activity was corrected for both protein content and Renilla activity to account for cell density and transfection efficiency, respectively. Primary rat hepatocytes were transfected using Lipofectamine 2000 (Invitrogen) as we have done previously (28). Transfections included 1 μg of CPT-Iα-luciferase and 0.1 μg of TK-Renilla. Luciferase assays were performed exactly as described above. COS-7 cells were transfected with 1.5 μg of Gal4-PGC-1α using Lipofectamine 2000.

Construction of the CPT-Iα-luciferase vectors was described previously (10). The Gal4-PGC-1α expression vectors were created by generating fragments of the PGC-1α cDNA by PCR amplification. The forward primers contained a BamHI restriction site, and the reverse primers contained an MluI restriction site. The PCR products were cut using BamHI and MluI and ligated into the MluI/MluI sites of the pCMV-Luc vector (BD Biosciences). The forward primer for the 5′-truncated Gal4-PGC-1α (1–140) was ggtggagtctgtggtggagaagttgccag, the following reverse primers were used: PGC-1α (1–140), gceaceggtacgttggctttggagtctgtggtggagaagttgccag; PGC-1α (1–210), gceaceggtacgttggctttggagtctgtggtggagaagttgccag; and PGC-1α (1–797), gceaceggtacgttggctttggagtctgtggtggagaagttgccag. The forward primers for the 5′-truncated PGC-1α (200–797) was ggtggagtctgtggtggagaagttgccag. The sequence of all vectors was confirmed by DNA sequencing at the University of Tennessee Molecular Resource Center.

Real Time PCR—RNA was extracted from primary rat hepatocytes using RNA-Stat-60 (Tel-Test) as described previously (9). The mRNA abundance was measured by real time PCR. The RNA was initially treated with DNase I (2 units) at 37 °C for 40 min. DNase I was stopped by addition of DNase Inactivation Reagent. Equal amounts of DNA-free RNA were used for first-strand cDNA synthesis. RNA was mixed with 1 μl of 10 mM dNTP mix and 1 μl of random hexamers (50 ng/μl). Each sample was incubated at 70 °C for 10 min and then placed on ice for at least 1 min. Next, 2 μl of 10× RT buffer, 4.5 μl of 25 mM MgCl₂, 2 μl of 0.1 M dithiothreitol, and 1 μl of RNaseOUT Recombinant Ribonuclease Inhibitor was added to each tube. After being incubated at 25 °C for 2 min, each tube was loaded with 1 μl of SuperScript II RT. The tubes were incubated at 25 °C for 10 min, at 42 °C for 1 h, and at 70 °C for 15 min. RNase H was added to each tube and incubated at 37 °C for 20 min.

![Table 1](https://example.com/table1.png)

**TABLE I**

The following primers were used for real time PCR to quantitate mRNA abundance

| Gene     | Primer 1                     | Primer 2                     |
|----------|------------------------------|------------------------------|
| CPT-I    | GAGAGCTTCGAGGATTTGTTG        | GAAGGAGTCTGTTACCACTGTCAG     |
| PGC-1α   | AAAGCAGTCTCAAGTGCGGT         | GAGAGCAATGGCAGGGTTTGTTC      |
| CPT-I    | CCGTACAAAGATGGCATGTCATC     | AAACAATCAGTGCACCGACAGT      |
| CPT-Iα   | TCACACCACACACACAGAGT        | GGCGCAGAAGATTTCAAGAT       |
| CPT-Iα   | GAGCGGTACCACTACGAAAAAGAA    | GGCTTGTCACTACTCCTCCCC      |

**TABLE II**

The following primers were used for the ChIP assay to demonstrate interactions of PGC-1α with the CPT-Iα gene

| Primer Set | Forward Primer | Reverse Primer |
|------------|----------------|----------------|
| Rat CPT-Iα TRE primers | GAACGACGGGTGATCATTTCAACAG | CAGGACATGTGTTTTTCTACAG |
| Rat CPT-Iα intron 1 primers | AAAGCAGTCTCAAGTTCGCGGT | GAGAGCTTCGAGGATTTGTTG |
| Rat CPT-Iα upstream promoter primers | CTCTGGTGTCCTGTAACCTGTGG | GAAGGAGTCTGTTACCACTGTCAG |
| Rat PEPCK primers | AACTCGCTGGTTGGCAGCCTT | CATCAGCAACAGTCAGGTC |

**FIG. 1.** Thyroid hormone increases PGC-1α mRNA and protein abundance in hepatocytes. A, primary rat hepatocytes were plated on collagen-coated plates for 16 h. T3 was added at a concentration of 100 nM to the hepatocytes for 24 h. RNA was harvested, and the abundance of PGC-1α and CPT-Iα mRNA determined by real time PCR was described under “Materials and Methods.” The data are presented as the fold induction of mRNA abundance by T3, and the control samples were assigned a relative value of 1. The numbers represent the average ± S.E. from four independent hepatocyte preparations. B, T3 was added at a concentration of 100 nM to rat hepatocytes for 24 h as described above. Cell lysates were prepared and analyzed for PGC-1α and actin abundance by Western blot analysis using antibodies as described under “Materials and Methods.” The abundance of PGC-1α was compared with actin which was used as a loading control. The numbers represent the average ± S.E. from four independent determinations. C, a representative Western blot is shown. The PGC-1α and actin immunoreactive proteins are indicated.
**Western Analysis**—Western analysis was performed on whole cell extracts prepared by lysis in PD buffer (40 mM Tris-Cl, pH 8.0, 500 mM NaCl, 0.5% Nonidet P-40, 6 mM EDTA pH 8.0, 6 mM EGTA, pH 8.0, 1 mM dithiothreitol, and diluted protease inhibitor mixture; P8340, Sigma). The cells were sonicated five times at a setting of 5 in a 100-watt Microson sonicator (Misonix XL2000). The cell membranes were removed by centrifugation for 25 min at 4 °C. An equal amount of protein was loaded onto a 12% SDS-PAGE gel and transferred to a pure nitrocellulose membrane (Bio-Rad). Blots were immunoreactive proteins. Actin was used as the loading control for each lane. The primary antibody was monoclonal mouse anti-actin IgM, and goat anti-mouse IgM was the secondary antibody (anti-PGC-1α, sc-13067, Santa Cruz Biotechnology). The second antibody was a goat anti-mouse IgG horseradish peroxidase conjugate (170-6156, Bio-Rad).

**Chromatin Immunoprecipitation (ChIP) Assays**—The ChIP assays were performed as follows. Rat primary hepatocytes were prepared as described previously (28). Rat primary hepatocytes (3 × 10⁶ in 60-mm dishes) were maintained for 12 h in priming media of RPMI 1640 and charcoal-stripped 10% fetal bovine serum (28). The cells were treated with 100 nM T3 for 24 h. Cross-linking was performed with 1% formaldehyde for 15 min at room temperature and stopped by adding 125 mM glycine to each plate for 5 min. Cross-linked hepatocytes were washed twice with ice-cold phosphate-buffered saline. Cells were scraped from the plate and collected by centrifugation for 5 min at 2,000 rpm. The cell pellets were resuspended in 200 μl of cell lysis buffer (50 mM Tris-HCl, pH 8.0, 85 mM KCl, 0.5% Nonidet P-40) containing protease inhibitor mixtures (P8340, Sigma). Samples were incubated on ice for 30 min and vortexed once, and the nuclear pellet was collected by centrifugation again at 4000 rpm for 5 min. Then nuclear pellets were resuspended in 200 μl of SDS lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris-HCl, pH 8.1) with protease inhibitor mixtures and incubated on ice for 10 min. The pellets were sonicated nine times for 30 s at 1-min intervals (with Branson sonifier 220-watt, 2-mm microtip setting 7), followed by centrifugation at 14,000 rpm for 10 min. The supernatant containing the DNA-protein complex was diluted 10-fold in ChIP dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl, pH 8.1, 167 mM NaCl) with protease inhibitors. The samples were pre-cleared with 80 μl of salmon sperm DNA/protein A-agarose, 50% slurry (16-157, Upstate Biotechnology, Inc.) for 30 min at 4 °C with rotation. After a brief centrifugation, the supernatant was transferred to a new tube. The supernatant was incubated with 5–10 μg of anti-PGC-1α (sc-13067, Santa Cruz Biotechnology), anti-acetylated histone H4 (06-599, Upstate Biotechnology, Inc.) or anti-acetylated histone H4 (06-599, Upstate Biotechnology, Inc.) at 4 °C overnight. Rabbit IgG (sc-2027, Santa Cruz Biotechnology) was used as the control. To collect the antibody-protein-DNA complex, 60 μl of protein A-agarose slurry was added for 2 h at 4 °C.

The protein A-agarose was washed one time with 1.0 ml of each of the following buffers: low salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 150 mM NaCl); high salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 500 mM NaCl); LiCl wash buffer (0.25 M LiCl, 1% Nonidet P-40, 1% deoxycholate, 1 mM EDTA, 10 mM Tris-HCl, pH 8.1). Following the washes, DNA was eluted from the agarose using 150 mM NaCl, followed by two 15-min washes with 1% SDS, 1% Triton X-100, 150 mM NaCl. The precipitated DNA was then extracted with chloroform:isoamyl alcohol (24:1 v/v) and ethanol-precipitated (27).
RESULTS

Our first experiments examined the effect of thyroid hormone (T3) on the abundance of PGC-1α mRNA in primary rat hepatocytes. Primary rat hepatocytes were treated with T3 for 24 h and then RNA was isolated. The mRNA abundance was determined by real time PCR. As can be seen in Fig. 1A, the PGC-1α mRNA abundance was increased 4.5 ± 0.4-fold by T3 in hepatocytes, and the CPT-Iα mRNA abundance was increased 3.3 ± 0.4-fold by T3. We also examined the effect of T3 on PGC-1α mRNA abundance in neonatal rat cardiac myocytes. Previously, we had found that CPT-Iα mRNA abundance was not increased in the heart of hyperthyroid rats (5). The CPT-Iβ protein abundance was increased in response to T3 administration in ventricular myocytes. In addition, PGC-1α levels did not change in the ventricular myocytes (data not shown). These data indicate that T3 can increase PGC-1α mRNA abundance in the liver but not in the heart and suggest that T3 induces PGC-1α in a tissue-specific manner. Next, we tested whether PGC-1α protein abundance was increased in response to T3 administration in rat hepatocytes. By using Western analyses to assess PGC-1α levels, we found that the PGC-1α protein abundance was increased 2.3 ± 0.3-fold in response to T3 (Fig. 1B). These experiments indicate that the PGC-1α is a T3-responsive gene and raise the possibility that it is a coactivator in T3 action.

and CPT-Iα mRNA levels were not altered by T3 administration in ventricular myocytes. In addition, PGC-1α levels did not change in the ventricular myocytes (data not shown). These data indicate that T3 can increase PGC-1α mRNA abundance in the liver but not in the heart and suggest that T3 induces PGC-1α in a tissue-specific manner. Next, we tested whether PGC-1α protein abundance was increased in response to T3 administration in rat hepatocytes. By using Western analyses to assess PGC-1α levels, we found that the PGC-1α protein abundance was increased 2.3 ± 0.3-fold in response to T3 (Fig. 1B). These experiments indicate that the PGC-1α is a T3-responsive gene and raise the possibility that it is a coactivator in T3 action.
FIG. 5. Amino terminus and LXXLL motif of PGC-1α participate in the thyroid hormone induction of CPT-Iα. HepG2 cells were transfected with 2 μg of −4495/+1240 CPT-Iα-luc, 1 μg of Gal4-PGC-1α, 1 μg of RSV-TRβ and TK-Renilla into HepG2 cells. T3 was added at a concentration of 100 nM for 24 h. The cells were harvested and assessed for luciferase and Renilla activity. The data are expressed as induction of luciferase by T3 or PGC-1α relative to control. All transfections were performed in duplicate and repeated 4–6 times. RXXR, retinoid X receptor; aa, amino acids.

Our next experiments examined the ability of PGC-1α to enhance the T3 induction of CPT-Iα. We conducted cotransfections of CPT-Iα-luc and pSV-PGC-1α in HepG2 hepatoma cells. By using transient transfections in HepG2 cells, we had previously found that the first intron was required for the full induction by T3 of the CPT-Iα gene (10). These results were confirmed by introducing CPT-Iα luciferase vectors with or without the first intron into transgenic mice (12). HepG2 hepatoma cells were transfected with −4495/+1240 CPT-Iα-luc or −4495/+19 CPT-Iα-luc. The −4495/+1240 luciferase vector contains the first intron and a portion of exon 2, whereas the −4495/+19 luciferase vector does not (Fig. 2). Addition of T3 stimulated the −4495/+1240 CPT-Iα-luc gene 8.2 ± 0.6-fold. In these experiments, overexpression of PGC-1α induced the CPT-Iα-luc vector 8.6 ± 0.9-fold. When T3 was added in the presence of PGC-1α, we obtained a 40.6 ± 6.2-fold induction. Without the intron, PGC-1α was only able to increase −4495/+19 CPT-Iα-luc 1.4 ± 0.2-fold, and the T3 induction was reduced by half. In the absence of the intron, T3 and PGC-1 stimulate the CPT-Iα-luc vector additively. These data indicate that PGC-1α stimulates CPT-Iα primarily through the first intron but, more importantly, that PGC-1α is a coactivator for the T3 induction of the CPT-Iα gene. The first intron is required for both the T3 and PGC-1α effects on CPT-Iα. We ligated two copies of an idealized TRE in front of the enhancerless SV40 promoter. PGC-1α induced this reporter vector modestly and enhanced the strong induction by T3.

To test whether PGC-1α also enhanced the T3 response in hepatocytes, we conducted transfections in primary rat hepatocytes. In the absence of cotransfected TRβ, T3 induced the −4495/+1240 CPT-Iα-luc vector 3.6 ± 0.3-fold (Fig. 3). Overexpression of PGC-1α only increased the reporter gene 1.9 ± 0.5-fold. PGC-1α may have a larger effect on basal expression of CPT-Iα-luc in HepG2 cells because HepG2 cells do not express PGC-1α. Most interestingly, when T3 was added in the presence of PGC-1α, the luciferase gene was synergistically induced 19 ± 4.5-fold. These results confirm our hypothesis that PGC-1α is a coactivator in the thyroid hormone regulation of CPT-Iα. Overexpression of TRβ slightly increased the T3 induction of CPT-Iα-luciferase to 4.4 ± 0.3, but the stimulation by T3 and PGC-1α was increased to 38.1 ± 10.1 (Fig. 3). These data demonstrate that T3 and PGC-1α act synergistically to stimulate CPT-Iα gene expression in both HepG2 cells and rat hepatocytes.

Our next experiments were designed to identify domains of PGC-1α that were required for the T3 stimulation of gene expression. Our initial experiments defined important domains for the stimulation of basal expression. We created a series of mammalian expression vectors with PGC-1α ligated to the Gal4 DNA binding domain. In Fig. 4A, we transfected 50 ng of Gal4-PGC-1α with a luciferase reporter containing five Gal4-binding sites. The Gal4-PGC-1α-(1–140) and Gal4-PGC-1α-(1–210) strongly induced expression, whereas the longer PGC-1α vectors had less effect on basal expression. These observations confirm previous reports that the first 120 amino acids of PGC-1α contain a strong transcriptional activation domain and that there is a transcriptional repression domain between amino acids 200 and 400 (20). We also altered the LXXLL motif found in amino acids 142–146 from LKKLL to AKKAL in the PGC-1α-(1–210) vectors. The motif is important for the interaction of PGC-1α with nuclear receptors including the TRβ, but these alterations did not reduce the ability of PGC-1α to stimulate the Gal4 reporters. To determine whether the Gal4-PGC-1α vectors were equally expressed, we harvested RNA from transfected HepG2 cells, and we measured the abundance of the Gal4 fusion protein mRNA using real time PCR. The relative expression of mRNA for the vectors was Gal4 (0.13), Gal4-PGC-1α-(1–140) (0.1), Gal4-PGC-1α-(1–210) (0.13), Gal4-PGC-1α-(1–410) (0.11), Gal4-PGC-1α-(1–797) (0.09), and Gal4-PGC-1α-(200–797) (0.08). These results indicate that the Gal4 vectors were expressed at relatively similar levels. In Fig. 4B, we show a Western blot conducted with an antibody to Gal4 on lysates of COS-7 cells transfected with the various Gal4 vectors. This analysis indicates that all the Gal4 fusion pro-
Teins generate proteins of the correct molecular weight and are expressed at similar levels.

To determine which domains of PGC-1α/H9251 were required for the T3 induction of the CPT-I/H9251 gene, we transfected CPT-I/H9251-luc with the Gal4-PGC-1/H9251 expression vectors and RSV-TRE/H9252 (Fig. 5). The Gal4-PGC-1-H9251-(1–797) induced CPT-I/H9251-luc in a manner that was essentially the same as pSV-PGC-1. Most interestingly, PGC-1α-(1–140) was not able to induce basal expression of CPT-I/H9251-luc indicating that other portions of the PGC-1α interacted with factors in the first intron and promoter. PGC-1α-(1–210) increased basal and T3 responsiveness demonstrating that the first 210 amino acids of PGC-1α are of particular importance in the regulation of CPT-Iα gene expression. Mutation of the LXXLL motif decreased the ability of PGC-1α-(1–210) to induce basal transcription and also reduced the synergistic effect of T3 and PGC-1α. Similar results were observed with the full-length PGC-1α-(1–797). Overall, these results show that the LXXLL motif is involved in recruiting PGC-1 to the CPT-Iα gene and that the first 210 amino acids of PGC-1 are sufficient for mediating the basal and T3 induction of CPT-Iα.

To determine whether PGC-1α was associated with the CPT-Iα gene in vivo, we conducted ChIP assays (Fig. 6). In these experiments, we used primary rat hepatocytes because we had cloned the rat CPT-Iα gene (9). Rat hepatocytes were exposed to T3 for 24 h or left in serum-free media. We cross-linked the hepatocytes with 1% formaldehyde prior to conducting immunoprecipitations with the PGC-1α antibody. As controls, we immunoprecipitated with rabbit pre-immune serum. We created PCR primers sets for the first intron, the TRE region, and an upstream promoter region of the CPT-Iα gene (Table II). The upstream region was a negative control. We also used the PEPCK gene as a positive control because it had been shown to recruit PGC-1α (29). Our ChIP assay indicated that prior to T3 treatment PGC-1α is associated with the first intron and the TRE region of the CPT-Iα gene around the TRE. However, it is not associated with the upstream region of the CPT-Iα gene. Addition of T3 to hepatocytes increased the association of PGC-1α with the first intron (2.6 ± 0.5-fold) and the TRE region (4.5 ± 2.0-fold) (Fig. 6). These data demonstrate that PGC-1α is a bona fide coactivator in the T3 response. The interaction of PGC-1α with the PEPCK promoter was elevated 2.0 ± 0.5-fold by T3. These data suggest that PGC-1α participates in the thyroid hormone induction of the PEPCK gene.

The induction of gene expression is frequently associated with an increase in histone acetylation (14). By using the ChIP assay, we examined whether there was an increase in histone...
Acetylation in the presence of T3. Immunoprecipitations were conducted with antibodies to acetylated histone 3 or acetylated histone 4 were used for immunoprecipitations. The location of the primers is shown schematically at the top of Fig. 6. The ChIP results using primers for the three regions of the CPT-Iα gene are shown. The gene for PEPCK was included as a positive control. These experiments were repeated four times. The relative abundance of the PCR products from the immunoprecipitates of untreated and T3-treated cells was determined. These data are presented at the bottom of the figure and represent the average of four independent experiments.

**Fig. 7.** Thyroid hormone increases histone acetylation of the CPT-Iα gene. ChIP assays were conducted on primary rat hepatocytes as described in Fig. 6. Antibodies to acetylated histone 3 or acetylated histone 4 were used for immunoprecipitations. The location of the primers is shown schematically at the top of Fig. 6. The ChIP results using primers for the three regions of the CPT-Iα gene are shown. The gene for PEPCK was included as a positive control. These experiments were repeated four times. The relative abundance of the PCR products from the immunoprecipitates of untreated and T3-treated cells was determined. These data are presented at the bottom of the figure and represent the average of four independent experiments.

**DISCUSSION**

In these studies, we have examined the role of PGC-1α as a coactivator in thyroid hormone responsiveness. T3 is a prominent regulator of CPT-Iα gene expression and activity in the liver (7, 26). We have provided multiple lines of evidence indicating that PGC-1α participates in the regulation of CPT-Iα gene expression by T3. Our data demonstrate that T3 increases the abundance of PGC-1α so that more PGC-1α will be available to stimulate the CPT-Iα gene. Our transient transfection experiments indicate that T3 will induce the CPT-Iα gene more effectively when PGC-1α is overexpressed. We have found that the *in vivo* association of PGC-1α with the CPT-Iα gene is increased by T3. PGC-1 may utilize all these mechanisms in the T3 induction of CPT-Iα and other hepatic genes. Although it has been known that PGC-1α will interact with the TR, to our knowledge CPT-Iα is the first gene that has been identified for which PGC-1α is involved in the T3 action (24).

Our first experiments examined the regulation of PGC-1α mRNA and protein abundance in primary cells. Previous studies (26, 30) from the Weitzel laboratory had identified PGC-1α and *NRF-1* as genes that were rapidly induced in the liver by T3. The authors gave T3 to hypothyroid rats and observed a 12-fold induction of PGC-1α mRNA abundance 6 h after T3 administration (26). Irrcher *et al.* (31) reported that administration of a lower dose of T3 to euthyroid rats increased PGC-1α protein abundance 1.5-fold in the liver after 5 days. They did not observe an increase in PGC-1α protein at 6 h (31). Overall, these data suggest that PGC-1α is elevated in the liver of hyperthyroid animals. Our data are the first to demonstrate that T3 directly increases PGC-1α mRNA and protein abundance in isolated hepatocytes. In our hepatocytes, which were
prepared from euthyroid animals, we found that T3 induced PGC-1α mRNA abundance 4.5-fold and PGC-1α protein 2.3-fold. The fact that T3 can increase PGC-1α levels suggests that T3 could induce PGC-1α responsive genes that do not contain a TRE in the promoter and suggests a mechanism for indirect actions of T3. Most interestingly, Irrcher et al. (31) reported that T3 did not increase PGC-1α abundance in the heart of rats treated with T3, although PGC-1α levels were increased in soleus muscle. This observation is consistent with our results that PGC-1α mRNA abundance was not increased in primary ventricular myocytes that were treated with T3 (data not shown). Although T3 will increase the expression of numerous cardiac genes, it does not appear that the elevation of PGC-1α is a mechanism for T3 action in the heart.

In addition to T3, other hormones will induce PGC-1α mRNA levels in the liver. It was reported that administration of cAMP and glucocorticoids to the hepatocytes will increase PGC-1α mRNA levels (27, 29, 32). Most interestingly, insulin did not decrease the abundance of PGC-1α mRNA (29). PGC-1α stimulates hepatic gluconeogenesis by increasing the expression of the PEPCK and G6P genes (21, 23). By using ChIP assays, it has been shown that PGC-1α is associated with the PEPCK promoter following the addition of cAMP and glucocorticoids, whereas the addition of insulin blocks this association (29). Our ChIP assays indicate that PGC-1α is associated with the PEPCK promoter prior to hormonal induction. Addition of T3 will recruit PGC-1α to the PEPCK promoter suggesting that PGC-1α will contribute to the elevated PEPCK expression observed in hyperthyroidism (33).

The CPT-Iα gene has a unique architecture in that two widely separated regions of the CPT-Iα gene participate in the regulation of the CPT-Iα by T3 (10, 12). The TRE in the promoter of the rat CPT-Iα gene is located almost 3000 bp 5′ to the start site of transcription (10). This region of the CPT-Iα promoter constitutes an important regulatory domain for the CPT-Iα gene, as there is an HNF-4-binding site (∼2867/∼2855) within 50 bp of the TRE. The TRE and HNF-4 sites are contained within a DNAse I-hypersensitive region in the CPT-Iα promoter (27). Both TRβ and HNF-4 could participate in recruiting PGC-1α to the CPT-Iα promoter. It has been reported that the liganded TRβ can interact physically with PGC-1α, and this interaction requires both the AF-2 domain and helix one of the TRβ (24). PGC-1α interacts with HNF-4 in a ligand-independent manner (22). In our ChIP assays, PGC-1α was associated with the CPT-Iα promoter in the absence of T3, and it may be recruited to the promoter by HNF-4. The interaction of HNF-4 and PGC-1α is important in the stimulation of the PEPCK and Glc-6-P genes (22, 23). It has been reported that PGC-1α can stimulate CPT-Iα through the HNF-4 binding site in the promoter although these studies were conducted primarily with small fragments of the promoter ligated in front of TK-luciferase (27). In the CPT-Iα gene, the first 210 amino acids of PGC-1α were required for both the enhancement of the T3 induction and stimulation of basal expression. The LKKLL motif in PGC-1α that interacts with the TR and HNF-4 is within amino acids 142–146 of the amino terminus. Our data demonstrate this motif is important for the recruitment of PGC-1α to the CPT-Iα promoter as the basal induction of CPT-Iα by PGC-1α is eliminated by disruption of this motif (Fig. 5). However, mutation of the LXXLL motif reduced but did not eliminate the ability of PGC-1α to potentiate the T3 induction of CPT-Iα (Fig. 5). PGC-1α can also interact with helix one of the TRβ, and this interaction may be sufficient to sustain partial activity of the CPT-Iα gene (24).

A unique feature of the CPT-Iα gene is the involvement of the first intron in the T3 induction. Deletion of the first intron reduces the stimulation by both T3 and PGC-1α. There are no TRES in the intron, and the intron appears to confer liverspecific responsiveness to T3 (12). We have identified several sites in the CPT-Iα intron that are required for the T3 induction (12). In addition, multiple sites in the first intron are required for the induction by PGC-1α (34). To date, we have been unable to identify any specific factors in the first intron that interact with PGC-1α, although it continues to be an area of investigation. In agreement with our transfection data, our ChIP assays demonstrate that PGC-1α interacts strongly with the first intron of the CPT-Iα gene. Our transfection results from Fig. 5 indicate that the LXXLL motif between 140 and 210 of PGC-1α is required for stimulating basal CPT-Iα gene expression. Our data do not distinguish whether individual PGC-1α molecules are associated with the promoter and first intron or whether there is looping between the intron and promoter and a single PGC-1α is a component in a bridge between these regions.

With the addition of T3, we found that there was increased acetylation of histone 3 and histone 4 in the CPT-Iα gene. Previous studies (14, 35) have indicated that the unliganded TR will bind DNA in association with corepressors such as NCoR and others. The addition of T3 leads to the recruitment of various families of coactivators including SRC, CREB-binding protein CBP/p300, and thyroid receptor activator proteins (TRAP220) (14). The SRC, CBP/p300, and pCAF proteins have histone acetyltransferase activity (13). PGC-1α does not have histone acetyltransferase activity suggesting that other coactivators may also be recruited to the CPT-Iα promoter. PGC-1α can interact with SRC-1, CBP, and TRAP220 raising the possibility that multiple coactivators are associated with the promoter simultaneously (37, 38). Other coactivators including CBP and SRC-1 participate in both the glucocorticoid and CAMP induction of PEPCK (36, 39). Future studies will examine the participation of other coactivators in the regulation of the CPT-Iα gene.

In summary, we have shown that T3 can induce the expression of PGC-1α in the liver. In addition, PGC-1α can stimulate CPT-Iα gene expression and enhance the induction by T3. Finally, the association of PGC-1α with the CPT-Iα and PEPCK genes in vivo is enhanced by T3. Overall, these results indicate that PGC-1α is a coactivator in the T3 induction of CPT-Iα in the liver and raises the possibility that PGC-1α is a general participant in the induction of hepatic genes in hyperthyroidism.

REFERENCES
1. McGarry, J. D., and Evans, N. F. (1997) Eur. J. Biochem. 244, 1–14
2. Cook, G. A., and Park, E. A. (1999) Amer. J. Med. Sci. 318, 43–48
3. Sorenson, A., Travers, M. T., Vernon, R. G., Price, N. T., and Barber, M. C. (2002) Brain Res. Gene Expr. Patterns 3, 167–173
4. Lavrentyev, E. N., Matta, S. G., and Cook, G. A. (2004) Biochem. Biophys. Res. Commun. 315, 174–178
5. Cook, G. A., Edwards, T. L., Jansen, S. M., Babouth, S. W., Wilcox, H., and Park, E. A. (2001) J. Mol. Cell. Cardiol. 33, 317–329
6. McGarry, J. D. (1992) Science 256, 766–770
7. Mynatt, R. L., Park, E. A., Thorngrate, F. E., Das, H. K., and Cook, G. A. (1994) Biochem. Biophys. Res. Commun. 201, 932–937
8. Park, E. A., Mynatt, R. L., Cook, G. A., and Kashtan, K. (1995) Biochem. J. 310, 853–858
9. Park, E. A., Steffen, M. L., Song, S., Park, V. M., and Cook, G. A. (1998) Biochem. J. 329, 217–224
10. Jansen, S. M., Cook, G. A., Song, S., and Park, E. A. (2000) J. Biol. Chem. 275, 34989–34997
11. Barrero, M. J., Marrero, P. F., and Haro, D. (2000) Amer. J. Med. Sci. 319, 61–88
12. Jackson-Hayes, L., Song, S., Lavrentyev, E. N., Jansen, S. M., Hillgarter, F. B., Tian, L., Wood, P. A., Cook, G. A., and Park, E. A. (2005) J. Biol. Chem. 278, 7964–7972
13. Rosenfeld, M. G., and Glass, C. K. (2001) J. Biol. Chem. 276, 36865–36868
14. Zhang, J., and Lazar, M. A. (2000) Annu. Rev. Physiol. 62, 439–466
15. Puigserver, P., Wu, Z., Park, C. W., Graves, R., Wright, M., and Spiegelman, B. M. (1998) Cell 95, 829–839
16. Puigserver, P., and Spiegelman, B. M. (2003) Endocr. Rev. 24, 78–90
17. Lin, J., Puigserver, P., Donovan, J., Tarr, P., and Spiegelman, B. M. (2002) J. Biol. Chem. 277, 1645–1648
PGC-1α Participates in Thyroid Hormone Induction of CPT-Iα

18. Knutti, D., Kaul, A., and Kralli, A. (2000) Mol. Cell. Biol. 7, 2411–2422
19. Lehman, J. J., Barger, P. M., Kovač, A., Saffitz, J. E., Medeiros, D. M., and Kelly, D. P. (2000) J. Clin. Invest. 106, 847–856
20. Vega, R. B., Huss, J. M., and Kelly, D. P. (2002) Mol. Cell. Biol. 20, 1868–1876
21. Yoon, J. C., Puigserver, P., Chen, G., Donovan, J., Wu, Z., Rhee, J., Adelmant, G., Stafford, J., Kahn, C. R., Granier, D. K., Newgard, C. B., and Spiegelman, B. M. (2001) Nature 413, 131–138
22. Rhee, J., Inoue, Y., Yoon, J. C., Puigserver, P., Fan, M., Gonzalez, F. J., and Spiegelman, B. M. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 4012–4017
23. Boustead, J. N., Stadelmaier, B. T., Eeds, A. M., Wiebe, P. O., Svitek, C. A., Oeser, J. K., and O’Brien, R. M. (2003) Biochem. J. 369, 17–22
24. Wu, Y., Delerive, P., Chin, W. W., and Burris, T. P. (2002) J. Biol. Chem. 277, 8898–8905
25. Lin, J., Tarr, P. T., Yang, R., Rhee, J., Puigserver, P., Newgard, C. B., and Spiegelman, B. M. (2003) J. Biol. Chem. 278, 30843–30848
26. Weitzel, J. M., Radtke, C., and Seitz, H. J. (2001) Nucleic Acids Res. 29, 5148–5155
27. Louet, J. F., Hayhurst, G., Gonzalez, F. J., Girard, J., and Decaux, J. F. (2002) J. Biol. Chem. 277, 37991–38000
28. Deng, X., Cagen, L. M., Wilcox, H. G., Park, E. A., Raghunath, R., and Elam M. B. (2002) Biochem. Biophys. Res. Commun. 296, 256–262
29. Puigserver, P., Rhee, J., Donovan, J., Walkey, C. J., Yoon, J. C., Oriente, F., Kitamura, Y., Altun, J. D., Deng, H., Accili, D., and Spiegelman, B. M. (2003) Nature 423, 550–555
30. Weitzel, J. M., Hamann, S., Jauk, M., Lacey, M., Filholy, A., Radtke, C., Iwen, K. A., Kutz, S., Harneit, A., Lizardi, P. M., and Seitz, H. J. (2003) J. Mol. Endocrinol. 31, 291–303
31. Irrcher, I., Adhiketty, P. J., Sheehan, T., Joseph, A. M., and Hood, D. A. (2003) Am. J. Physiol. 284, C1669–C1677
32. Herzg, S., Long, F., Jhala, U. S., Hedrick, S., Quinn, R., Bauer, A., Rudolph, D., Schutz, G., Yoon, C., Puigserver, P., Spiegelman, B. M., and Montminy, M. (2004) Nature 413, 179–183
33. Loose, D. S., Cameron, D. K., Short, H. P., and Hanson, R. W. (1965) Biochemistry 24, 4509–4512
34. Song, S., Zhang, Y., Ma, K., Jackson-Hayes, L., Lavrentyev, E. N., Cook, G. A., Elam, M. B., and Park, E. A. (2004) Biochim. Biophys. Acta 1679, 164–173
35. Weitzel, J. M., Iwen, K. A., and Seitz, H. (2003) Exp. Physiol. 88, 121–128
36. Stafford, J. M., Waltner-Law, M., and Granier, D. K. (2001) J. Biol. Chem. 276, 3811–3819
37. P. Puigserver, P., Adelmant, G., Wu, Z., Fan, M., Xu, J., O’Malley, B. M., and Spiegelman, B. M. (1999) Science 286, 1368–1371
38. Wallberg, A. E., Yamamura, S., Malik, S., Spiegelman, B. M., and Roeder, R. G. (2003) Mol. Cell 12, 1137–1149
39. Duong, D. T., Wakt tailor-Law, M. E., Sears, R., Sealy, L., and Granier, D. K. (2002) J. Biol. Chem. 277, 32234–32242
Peroxisomal Proliferator-activated Receptor-γ Coactivator-1α (PGC-1α) Enhances the Thyroid Hormone Induction of Carnitine Palmitoyltransferase I (CPT-Iα)

Yi Zhang, Ke Ma, Shulan Song, Marshall B. Elam, George A. Cook and Edwards A. Park

J. Biol. Chem. 2004, 279:53963-53971.
doi: 10.1074/jbc.M406028200 originally published online October 6, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M406028200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 39 references, 14 of which can be accessed free at
http://www.jbc.org/content/279/52/53963.full.html#ref-list-1