Upstream Stimulatory Factor Represses the Induction of Carnitine Palmitoyltransferase-1β Expression by PGC-1*

Transcriptional regulation of carnitine palmitoyltransferase-1β (CPT-1β) is coordinated with contractile gene expression through cardiac-enriched transcription factors, GATA4 and SRF. Metabolic modulation of CPT-1β promoter activity has been described with the stimulation of gene expression by oleate that is mediated through the peroxisome proliferator-activated receptor (PPAR) pathway. The coactivator, peroxisomal proliferator-activated receptor γ coactivator (PGC-1), enhances gene expression through interactions with nuclear hormone receptors and the myocyte enhancer factor 2 (MEF2) family. PGC-1 and MEF2A synergistically activate CPT-1β promoter activity. This stimulation is enhanced by mutation of the E-box sequences that flank the MEF2A binding site. These elements bind the upstream stimulatory factors (USF1 and USF2), which activate transcription in CV-1 fibroblasts. However, overexpression of the USF proteins in myocytes depresses CPT-1β activity and significantly reduces MEF2A and PGC-1 synergy. Co-immunoprecipitation studies demonstrate that PGC-1 and USF2 proteins can physically interact. Our studies demonstrate that PGC-1 stimulates CPT-1β gene expression through MEF2A. USF proteins have a novel role in repressing the expression of the CPT-1β gene and modulating the induction by the coactivator, PGC-1.

Carnitine palmitoyltransferase-1 (CPT-1) is located on the outer mitochondrial membrane and functions in the transport of fatty acids into the matrix for β-oxidation. The β isoform (CPT-1β) is primarily expressed in skeletal and cardiac muscle and white adipose tissue (1). The α isoform of CPT-1 (CPT-1α) predominates in the remaining tissues and exhibits different kinetics from the muscle isoform (1). Early evidence suggests that CPT-1β is transcriptionally up-regulated in response to electrical stimulation and consequent hypertrophic growth (2). The cardiac-enriched factors GATA4 and serum response factor (SRF) are synergistic activators of CPT-1β expression (3). Because metabolic substrate preference shifts with hypertrophy and failure, recent studies have focused on the identification of transcription factors capable of transmitting nutritional as well as pathological messages into changes in gene transcription.

Fatty-acid induction of gene expression through peroxisome proliferator activator receptor-α (PPARα) binding to the muscle-specific form of CPT-1β has been extensively studied (4, 5). However, the rat CPT-1β gene is only modestly induced by physiological levels of fatty acids (6), and CPT-1β message is increased 2-fold in rodent models of fasting and diabetes where circulating fatty acids are elevated (7). The PPARα-mediated regulation of several genes is enhanced by the PPARγ coactivator-1 (PGC-1). PGC-1 is highly expressed in metabolically active tissues including brown fat, skeletal muscle, and heart (8). PGC-1 has been implicated in mitochondrial biogenesis in the heart and increased mitochondrial respiration in brown fat (8). PGC-1 is a coactivator for many factors in the nuclear hormone receptor family including PPARα, the glucocorticoid receptor, the thyroid hormone receptor, and several orphan receptors (9–12). These combinatorial interactions upregulate the expression of fatty acid oxidation, oxidative phosphorylation, and tricarboxylic acid cycle enzymes as well as uncoupling proteins in response to cold, fasting, or exercise (8). PGC-1 also physically interacts with myocyte enhancer factor 2C (MEF2C) to upregulate GLUT4 expression and glucose uptake in L6 cells that were overexpressing PGC-1 (13). Associations with negative binding partners have been proposed but no specific proteins have yet been identified (14).

Upstream stimulatory factor (USF) is a member of the basic helix-loop-helix leucine zipper family and preferentially binds to the E-box consensus CANNTG with CG as interior nucleotides (15). In the heart, USF proteins regulate the expression of energy transfer and contractile-responsive sarcomeric genes (16). We have shown that USF heterodimers bind to two E-box sites within the CPT-1β promoter. However, the regulatory significance of USF in the context of this gene is unknown (3). Although USF is expressed ubiquitously, the relative amount of USF proteins varies with tissue type (17). Alterations in USF protein stoichiometry may be involved in regulating different sets of genes. While a high degree of amino acid conservation characterizes their dimerization and DNA binding sequences, their extreme divergence in N-terminal amino acid sequences could direct selective contacts for a variety of transcription factors (17).

Here, we have examined the regulation of the CPT-1β gene by PGC-1 and USF proteins. We found that PGC-1 stimulates CPT-1β expression through interactions with MEF2A. Furthermore, the induction by PGC-1 and MEF2A is inhibited by
USF. We demonstrate that PCG-1 and USF proteins can physically interact. Our results demonstrate that PCG-1 is a powerful stimulator of a key regulatory protein in long chain fatty acid oxidation in the heart and suggest a novel role for USF in modulating PCG-1 action on the CPT-1 β gene.

EXPERIMENTAL PROCEDURES

Plasmids and Luciferase Constructs—The p-391/+80 rat CPT-1 β luciferase construct has been described (McMillin et al., Ref. 18). Generation of site mutations within the −391/+80 fragment was performed with the QuickChange Mutagenesis kit (Stratagene, La Jolla, CA) as reported (3). All promoter constructs and their correct insertions were confirmed by DNA sequencing.

Cell Culture and Transfections—Isolation of neonatal rat cardiac myocytes was performed as previously described (19). Cells were plated in 6-well plates (Primera, Fisher, Pittsburgh, PA) at a cell density of 6 × 10^6 cells/well. Dulbecco’s Modified Eagle’s Medium was supplemented with 1% penicillin/streptomycin (Invitrogen) and 10% calf serum (Hyclone Laboratories, Inc., Logan, UT). Cultures were maintained at 37 °C in an atmosphere of 95% air and 5% CO_2 for 36 h before transfection. CV-1 fibroblast cells (ATCC no. CCL-70) were trypsinized and plated to 85% confluence. Both cell types were transfected with LipofectAMINE PLUS Reagent (Invitrogen) in serum-free medium for 3 h. The medium was then replaced with serum-containing culture medium for an additional 48 h. Transfections included 1.0 μg of wild-type or mutated CPT-1 β −391/+80 firefly luciferase reporter gene construct and 0.25 μg of pRL-CMV-Renilla luciferase construct to control for transfection efficiency. Co-transfections included various combinations of the following CMV-driven expression vectors: USF1, USF2, USF2ΔB or USF2ΔN (from Dr. M. Sawai-dago), and pSV-PGC-1 (from Dr. B. Spiegelman). Total DNA concentrations were kept constant with the corresponding empty vectors. Final protein concentrations were determined with the BCA protein assay (Pierce, Rockford, IL), and luciferase activities were measured with the Promega Dual Luciferase kit (Madison, WI). Promoter activity is expressed as Renilla-corrected firefly luciferase/total protein.

Electrophoretic Mobility Shift Assay—Nuclear extracts from primary rat neonatal myocytes were prepared as described (20). Double-stranded DNA probes that contained sequences from the rat CPT-1 β promoter were synthesized by Operon Technologies, Inc. (Alameda, CA) as follows: E-box-315 etagacagctacctagctacctggg (underlined sequences from E-box-315 were mutated to TagCTT); E-box-252 etagacatgtgcagacctg (underlined sequences from E-box-252 were mutated to ßCggCA). EMSA reaction mixtures included 2–5 μg of nuclear extract, 25 μl Hepes, 100 μM KCl, 0.1% Nonidet P-40 (v/v), 1 μM dithiothreitol, 5 μg/ml, and 50 pg of polydeoxycytidine-deoxyoctytosine (poly(dC-dC)] as a nonspecific competitor in a 20-μl reaction volume. After a 10-min room temperature incubation, 0.3 ng of radiolabeled probe was added, and the reaction was allowed to incubate for an additional 20 min. Antibody (Santa Cruz Biotechnology, Santa Cruz, CA) or USF-2 were expressed using a linked transcription/translation kit (PROTEInScrip II from Ambion) (22). GST-USF-1 proteins bound to Sepharose-4B were incubated with 35S-labeled proteins in 20 μL Tris-HCl (pH 8.0), 150 μM NaCl, 1 μM EDTA, and a protase inhibitor mixture (Sigma). The Sepharose-4B-bound GST proteins were washed four times with the buffer used for binding. Bound proteins were eluted in Laemmli buffer and resolved by SDS-PAGE. The 35S-labeled proteins were visualized by storage phosphor autoradiography.

RESULTS

Our first experiments examined the relative affinity of nuclear protein binding to the E-boxes in the CPT-1 β promoter. We have previously shown that USF1 and 2 bind to the E-box sequences from the CPT-1 β promoter as heterodimers (3). The consensus E-box element −252 robustly binds USF proteins (Fig. 1, lane 2). The E-box element −315 requires additional nuclear extract from cardiac myocytes to produce a similar band (Fig. 1, compare lane 2, 2-μl nuclear extract versus lane 8, 5-μl nuclear extract). To examine further the relative binding strengths of the two E-box regions, increasing concentrations of the unlabeled alternate DNA sequence were added to the gel shift binding reaction. Residual USF binding persists on element −252 even in the presence of 100-fold molar excess of the unlabeled −315 sequence (Fig. 1, lanes 3–5). However, a 25-fold excess of −252 competitor is sufficient to compete all protein binding to the E-box −315 (Fig. 1, lanes 9–11). Consistent with its consensus sequence, E-box −252 is the stronger USF binding site. However, E-box-315 also binds USF with lower affinity.

To define a regulatory role for USF proteins with the CPT-1 β gene, both USF1 and USF2 were transfected into CV-1 fibroblasts with the CPT-1 β luciferase reporter construct. A model of binding sites in the CPT-1 β promoter is shown in Fig. 2, top panel. Expression of the CPT-1 β promoter increased 15.8 ± 1.5-fold by USF overexpression (Fig. 2). Co-transfection of a vector with the N-terminal activation domain of USF2 deleted
and luciferase activity measured and normalized as described. USF1, USF2, USF2N, or USF2B as indicated. Cells were harvested, and luciferase activity measured and normalized as described. Bars represent the mean ± S.E. for triplicate measurements from three independent experiments. Inset, 10 μg of protein from CV-1 cells transfected with USF2, USF2ΔN, or USF2ΔB was separated on 10% ready gels and analyzed by Western blot with specific USF2 antibodies. *, p < 0.0005; §, p < 10⁻⁶.

(Fig. 2, USFΔN) significantly reduced USF1/USF2 up-regulation of the CPT-1β construct. Removal of the DNA binding/dimerization domain of USF2 (Fig. 2, USFΔB) diminished promoter activation by 6.5 ± 0.9-fold (p < 0.0001). A weaker functional inhibition by USFΔN compared with USFΔB may be, in part, due to reduced expression of the former construct in CV-1 cells (Fig. 2, inset). Therefore, USF proteins can activate CPT-1β in CV fibroblasts and requires intact protein structure to regulate gene expression.

Since cell-type dependent functions have been reported for USF1 and USF2 (23, 24), we wished to determine whether similar effects of USF-1 on CPT-1β would be observed in cardiac myocytes. We transfected out CPT-1β-luciferase into cardiac myocytes with the USF expression vectors. In cardiac myocytes, mutation of E-box –315 increases promoter activity 2.2 ± 0.2-fold (p < 0.01), suggesting that USF proteins function to suppress CPT-1β gene expression in this cell type. Consistent with this idea, overexpression of USF1 and USF2 in cardiac myocytes reduces CPT-1β luciferase expression to less than 40% of basal activity (Fig. 3). Substitution of a DNA binding-deficient mutant USF2 for the wild-type factor does not affect USF2-mediated suppression. However, truncation of the N-terminal domain returns luciferase values to baseline, suggesting that the suppressor activity of USF2 may be localized in this region (Fig. 3). The finding of opposite effects of USF overexpression in CV-1 fibroblasts versus cardiac myocytes suggests that USF-mediated repression of CPT-1β reporter gene expression requires USF interactions with myocyte-enriched factors.

PPARs and RXR can bind to the fatty acid response element (FARE) (also called PPRE) of CPT-1β, and these nuclear receptors can interact with PGC-1 (25, 26). In CPT-1β, the PPRE is flanked by the two E-box motifs, and this region also contains the myocyte-specific (MEF2F) site that binds MEF2A and MEF2C (3). To identify potential cell-specific interactions, we first investigated the effects of USF on the PGC-1 regulation of the CPT-1β promoter in neonatal cardiac myocytes. Transfection of PGC-1-stimulated reporter activity in cardiac myocytes in a dose-dependent manner (Fig. 4A). Overexpression of USF proteins in the myocyte culture completely blunted the PGC-1 induction of CPT-1β and returned expression to basal levels (Fig. 4B). To determine the interactions by which USF interferes with PGC-1 function in cardiac myocytes, we investigated this regulatory motif for myocyte-specific proteins, e.g. MEF-2, that are known to recruit the latter cofactor (18). Endogenous levels of muscle-specific transcription factors often interfere with transfection studies in myocytes or are already expressed at saturating concentrations. Therefore, to determine the magnitude of MEF2A and PGC-1 regulation of CPT-1β, these expression vectors were co-transfected into a cardiac-null fibroblast cell line, CV-1. Here, MEF2A synergistically and significantly co-activates PGC-1 induction of CPT-1β reporter gene expression by greater than 40-fold (Fig. 5). Because USF prevented PGC-1 activation of CPT-1β luciferase expression in cardiac myocytes (Fig. 4), we asked whether the bHLH factor could also interfere with PGC-1/MEF2A synergy. Addition of USF1 and 2 expression vectors abolished synergy and decreased PGC-1/MEF2A activation by greater than 60% (Fig. 5). Substitution of either serum response factor (SRF), or the cardiac-enriched factor, Nkx2.5, for USF does not affect PGC-1/MEF2A synergy (Table I). To determine if USF proteins might be interfering with the ability of MEF2A to stimulate CPT-1β, we cotransfected CPT-1β-Luc with expression vectors for USF proteins and MEF2A. USF1/2 alone stimulated −391/+80 CPT-1β-Luc 4.3 ± 0.6-fold, while MEF2A stimulated 1.7 ± 0.15-fold. When both USF and MEF2A were transfected, the CPT-1β-Luc vector was stimulated 6.1 ± 0.65-fold. These data indicate that USF represses CPT-1β promoter activity by blocking synergistic interactions between PGC-1 and the muscle-specific transcription factor, MEF2A.

Fig. 2. Domain deletions within the USF2 protein abrogate function in CV-1 cells. Top, diagram of the CPT-1β promoter is shown and the key regulatory elements identified. Early passage CV-1 fibroblasts were co-transfected with 1 μg each −391/+80 wild-type reporter, USF1, USF2, USF2ΔN, or USF2ΔB as indicated. Cells were harvested, and luciferase activity measured and normalized as described. Bars represent the mean ± S.E. for triplicate measurements from three independent experiments. Inset, 10 μg of protein from CV-1 cells transfected with USF2, USF2ΔN, or USF2ΔB was separated on 10% ready gels and analyzed by Western blot with specific USF2 antibodies. *, p < 0.0005; §, p < 10⁻⁶.

Fig. 3. Overexpression of USF proteins inhibits activity of the CPT-1β promoter construct. Myocytes were co-transfected as described with 1 μg of −391/+80 reporter gene construct. Combinations of 1.0 μg of USF1 and either 1.0 μg of USF2 (wild-type) or USF2ΔB or USF2ΔN (1 μg each) were overexpressed, as indicated below the x axis. Total DNA concentrations were kept constant with the empty vector pSG5. Cells were harvested after 48 h in serum containing Dulbecco’s modified Eagle’s medium. Luciferase was measured and corrected for Renilla expression and protein content. Bars represent mean ± S.E. for triplicate measurements from three separate passages. *, p < 10⁻⁵.
To confirm that E-box-binding proteins could alter PGC-1/MEF2A interactions, reporter constructs with mutations in either or both E-box elements were transfected with the synergistic combination into CV-1 cells. Individual E-box mutations in either E-box –315 or E-box –252 increased synergy to 69 ± 4- and 82 ± 7-fold, respectively (versus 43 ± 3-fold for wild type, Fig. 6). The double E-box mutant showed an even greater response to PGC-1/MEF2A, i.e. 117 ± 8-fold induction (Fig. 6). PGC-1/MEF2A synergistic activation of the double E-box mutant construct remained sensitive to USF overexpression (Table I), suggesting that direct DNA binding was not essential for USF modulation of PGC-1/MEF2A interactions. Therefore, based on these data, USF appears to function by regulating MEF2 recruitment of cofactors like PGC-1.

Our next experiments were performed to determine if USF proteins could interact with PGC-1. Co-immunoprecipitation experiments with anti-USF2 IP of neonatal rat cardiac myocyte extract revealed PGC-1 interactions following immunoblots with anti-PGC-1 antibodies (Fig. 7A, IP). Anti-USF2 IP of neonatal rat cardiac myocyte extract revealed PGC-1 interactions following immunoblot with anti-PGC-1 antibodies (Fig. 7A, IB). PGC-1 protein was also detected in control anti-MEF2 immunoprecipitates but not anti-actin precipitates (data not shown). To confirm USF2/PGC-1 interaction, anti-GST immunoprecipitation with PGC-1-GST fusion protein and USF2 TnT products also revealed the presence of USF2 protein (Fig. 7A). IP reactions with unpurified TNT products did not pull-down USF2 protein (data not shown). We also conducted GST-PGC-1 pull-down experiments using bacterially expressed GST-PGC-1 and [35S]methionine-labeled USF1 or USF2. As is shown in Fig. 7B, the full-length PGC-1 or PGC-1 vectors containing either amino acids 1–400 or 1–170 were able to pull-down USF1 and USF2. GST alone did not interact with either isoform of USF. These data indicate that USF proteins can interact with peptides within the first 170 amino acids of PGC-1.

### Discussion

Fatty acid oxidation accounts for the majority of energy utilization in the normal, beating heart and fatty acid is the dominant fuel used by the diabetic heart. Since CPT-1β regulates fatty acid entry into β-oxidation, this enzyme has a prominent role in modulating changes in fuel selection. Long term adaptive responses extend beyond localized adjustments in enzyme activity from changes in the malonyl-CoA pool. Metabolic up-regulation of CPT-1β gene expression by fatty acids occurs through the ligand-dependent activation of PPARα bind-

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**Table I**

| Transcription factor | Loss of synergy | p value |
|----------------------|----------------|---------|
| SRF                  | 19             | 0.23    |
| Nkx2.5               | 24             | 0.12    |
| USF (wild-type promoter) | 47            | <0.005  |
| USF (DEM promoter)   | 75             | <10^–5  |

**Inhibition of PGC-1/MEF2A synergy is specific for USF**

CV-1 fibroblasts were co-transfected with 1.0 μg of luciferase reporter, 0.5 μg of PGC-1, 0.5 μg MEF2A, and either 0.1 μg of SRF, 0.5 μg of Nkx2.5, or 0.25 μg each USF1 and USF2. USF inhibition studies were also performed on the double E-box mutant promoter (DEM). Data are expressed as percent reduction in the magnitude of synergistic activation compared to empty vector controls.

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**Fig. 4.** USF expression blunts PGC-1 up-regulation of the CPT-1β promoter construct. A, myocytes were co-transfected as described with 1 μg of −391/+ 80 reporter gene construct and increasing concentrations of PGC-1 expression vector, as indicated. B, myocytes were co-transfected with 1 μg of the −391/+ 80 CPT-1β construct and 0.5 μg each of USF1, USF2, and PGC-1 as indicated. Total DNA concentrations were kept constant with the appropriate empty vector. Cells were harvested after 48 h in serum containing Dulbecco’s modified Eagle’s medium. Luciferase was measured and corrected for Renilla expression and protein content. Bars represent mean ± S.E. for triplicate measurements from 3 separate passages. *, p < 0.0001 compared to PGC-1 alone.

**Fig. 5.** PGC-1 and MEF2A synergistic activation of CPT-1β is sensitive to USF1/2 overexpression. Subconfluent CV-1 cells were transfected as described with 1 μg of reporter, 0.5 μg PGC-1, 0.5 μg MEF2A, and 0.25 μg each USF1 and USF2, as indicated. Total DNA concentrations were kept constant at 2.5 μg with the appropriate empty vector. Cells were maintained in serum-containing media for 48 h, harvested, and assayed as described. Bars represent triplicate measurements from three independent experiments presented as mean ± S.E. The significance of comparisons indicated as an asterisk (p < 10^–5) compared with PGC-1 alone, and § (p < 10^–5) compared with PGC-1 plus MEF2A.

**Fig. 6.** PGC-1/MEF2A synergy is enhanced by E-box sequence mutation. Early passage CV-1 cells were transfected with the synergistic combination 0.5 μg of PGC-1 and 0.5 μg of MEF2A with 1.0 μg of either wild-type or E-box mutant reporter construct as indicated (DEM, double E-box mutant). Cells were harvested after 48 h in serum containing Dulbecco’s modified Eagle’s medium, and luciferase was measured and corrected for both Renilla expression and protein content. Bars represent mean ± S.E. for at least three experiments performed in triplicate, and p values are compared with synergy on the wild-type promoter.
is a general phenomenon or is mediated in a gene-specific and tissue-specific manner.

The up-regulation of metabolic genes critical to mitochondrial energy production in the heart is controlled by factors binding to conserved regulatory motifs found in heart and striated muscle, including the GATA and MEF2 elements and the CArG and E-boxes. These motifs, present in the α myosin heavy chain gene (28), troponin I (29), α actin (30), muscle creatine kinase (31), and the Na−/Ca2+ exchanger (32), are also conserved in the muscle-specific mitochondrial isoforms of cytochrome oxidase (33), mitochondrial creatine kinase (34), and CPT-1β (18). These genomic sequences have been proposed to be necessary in specifying expression of genes involved in high energy phosphate production and energy channeling to the unique oxidative requirements of cardiac muscle (34).

In contrast to the normal working heart, carbohydrate utilization increases at the expense of lipids during cardiac hypertrophy. Hypertrophied hearts exhibit reduced levels of PPARα expression with a down-regulation of fatty acid oxidation genes (35). Therefore, maladaptive reliance on glycolytic pathways in heart failure and concomitant down-regulation of CPT-1β suggest that glucose-responsive transcription factors may play a role in apophasiological changes in fatty acid and glucose metabolism genes.

USF binding to E-box sites in cardiac-specific promoters has been proposed to be a component of protein complexes that coordinately control the expression of myosin light-chain 2 and α myosin heavy chain genes (36, 37). The role of internal and flanking E-box nucleotide sequences in the context of the cardiac Tns1 gene demonstrates that the specific nucleotide composition is important to the regulation of bHLH-mediated gene expression (29). Likewise, the majority of genes in which the promoter contains USF-binding E-boxes are not regulated by glucose (38). Thus, the context of the E-box and the protein composition of the USF-binding complex are important in eliciting a transcriptional response. USF is ubiquitously expressed (17), so tissue-specific factors should play an important role in determining the ultimate regulatory consequences of USF activity. We have demonstrated that the action of USF on CPT-1β reporter gene expression is dependent on cell-specific protein interactions, where USF up-regulates CPT-1β in fibroblasts and down-regulates CPT-1β in cardiac myocytes. PGC-1-mediated activation of CPT-1β is abolished by USF in cardiac myocytes as well as in fibroblasts when the cardiac context for the MEF2 element adjacent to E-252 on the CPT-1 gene is invoked by overexpression of the cardiac-specific factor, MEF2A.

PGC-1 is most widely recognized for its role in co-activating nuclear hormone receptors so that the downstream targets, while varied, are genes involved in thermogenesis, energy production, and mitochondrial biogenesis (8, 25, 39, 40). The inducible nature of PGC-1 expression provides the cell with a mechanism for stimulation of metabolism in response to stress (40). PGC-1-mediated up-regulation of GLUT4 responds to an increased need for metabolic substrate to prevent self-catabolism (13). This proposition could also be extended to include a possible role for the up-regulation of fatty acid import (27).

USF binding to E-boxes is required for transcriptional activation of a variety of cardiac genes including contractile proteins as well as for the activity of the α B-crystallin enhancer (41). Alteration in MAPK/ERK signaling pathways is a molecular mechanism by which factor phosphorylation, e.g. PPARα (35), may coordinate and modulate these various signaling pathways during hypertrophic growth. Phosphorylation of p38 enhances PGC-1 co-activation of PPARα (42, 43) and promotes transduction of cytokine signals to PGC-1 regulated genes (44). During contractile stimulation, USF1 is phosphorylated result-
ing in enhanced DNA binding and increased α myosin heavy chain promoter activity (45). Application of a phosphorylation-dependent regulatory scheme to PGC-1/USF interactions would identify another transcription factor interaction responsible for cross-talk among cardiac gene families. MAPK activity releases PGC-1 from the inhibitory association of a PGC-1/ repressor complex associated with the L3/L3 region of PGC-1 (14) and promotes co-activation of PGC-1-dependent promot-
ers. If PGC-1/USF association is regulated in a similar manner, this phosphorylation-dependent mechanism could potentially integrate PGC-1 stimulated fatty acid metabolism with in-
creased contractile gene expression. Alternatively, the MEF2 binding site flanked by E-box elements within the promoters of contractile genes (46–48) could also recruit modulatory USF proteins.

In summary, the current data suggests that PGC-1 will promote high levels of CPT-1β gene expression in the heart through interactions with PPARα and MEF2C. USF proteins can modulate the inductive effects of MEF2C and PGC-1. USF2 and PGC-1 physically interact, defining a new mechanism for the upstream stimulatory factors in the coordination of fatty acid oxidation genes. The potential exists for phosphorylation control to integrate stress kinase signaling with gene expression through these transcription factor interactions. Given the altered substrate preference and increased p38 activity in pathologic hypertrophy, this regulatory mechanism may play a significant role in the pleiotropic genetic response to disease.

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