p38 Mitogen-activated Protein Kinase Activates Peroxisome Proliferator-activated Receptor α

A POTENTIAL ROLE IN THE CARDIAC METABOLIC STRESS RESPONSE

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The expression of enzymes involved in fatty acid β-oxidation (FAO), the principal source of energy production in the adult mammalian heart, is controlled at the transcriptional level via the nuclear receptor peroxisome proliferator-activated receptor α (PPARα). Evidence has emerged that PPARα activity is activated as a component of an energy metabolic stress response. The p38 mitogen-activated protein kinase (MAPK) pathway is activated by cellular stressors in the heart, including ischemia, hypoxia, and hypertrophic growth stimuli. We show here that PPARα is phosphorylated in response to stress stimuli in rat neonatal cardiac myocytes; in vitro kinase assays demonstrated that p38 MAPK phosphorylates PPARα significantly enhanced ligand-dependent transactivation. Cotransfection studies performed with several known coactivators of PPARα demonstrated that p38 MAPK markedly increased coactivation specifically by PGC-1, a transcriptional coactivator implicated in myocyte energy metabolic gene regulation and mitochondrial biogenesis. These results identify PPARα as a downstream effector of p38 kinase-dependent stress-activated signaling in the heart, linking extracellular stressors to alterations in energy metabolic gene expression.

The expression of enzymes involved in fatty acid β-oxidation (FAO), the principal source of energy production in the adult mammalian heart, is tightly controlled at the transcriptional level during cardiac development and in response to physiologic and pathophysiologic stimuli (1–7). The nuclear receptor PPARα has been shown to serve as a key transcriptional regulator of this energy metabolic pathway (Ref. 8; reviewed in Ref. 9). PPARα is a member of the nuclear receptor superfamily of transcription factors and binds cognate response elements as an obligate heterodimer with the retinoid X receptor (RXR). PPARα is ligand-activated by a variety of natural and synthetic agonists, including arachidonic acid derivatives, fibrates, and long-chain fatty acids: metabolic substrates for cardiac FAO enzymes. The important role played by PPARα in cardiac metabolism is underscored by the marked reduction in the basal level of cardiac FAO enzyme gene expression in PPARα−/− mice (10, 11), leading to reduced long-chain fatty acid uptake and oxidation (12).

Evidence has emerged that PPARα plays a critical role in the energy metabolic stress response in tissues that rely largely on mitochondrial fat oxidation for energy production, such as heart and liver. Under normal physiologic conditions, the expression of cardiac FAO enzyme genes are induced after a short term fast coincident with increased use of fatty acids for myocardial energy production (1, 3). In contrast, PPARα−/− mice do not exhibit the expected fasting-mediated induction of most FAO enzyme genes, but instead develop hypoglycemia, exhibit inadequate ketogenesis, accumulate neutral lipid in both heart and liver, and have a high death rate relative to wild-type mice (1). In addition, metabolic inhibition experiments and studies of senescent PPARα−/− mice implicate PPARα in the cardiac and hepatic lipid homeostatic response (10, 13, 14). Finally, PPARα expression and activity are induced by physiologic stimuli known to increase energy demand and mitochondrial oxidative flux such as electrical activation of canine skeletal muscle (15) and in humans subjected to a course of endurance training (16). Taken together, these results suggest that PPARα serves as a metabolic stress response factor to transduce changes in cellular energy demand and fatty acid uptake into oxidative energy-producing capacity via the transcriptional control of FAO enzyme expression.

The response of the postnatal heart to growth and stress stimuli includes activation of a network of signal transduction cascades, including the stress-activated protein kinases, p38 mitogen-activated protein kinase (MAPK) and c-Jun NH2-terminal kinase (JNK) (reviewed in Refs. 17–19). Evidence is emerging that the p38 MAPK pathway is an important component of the cardiac cellular stress response. p38 MAPK is activated in heart and other tissues by inflammatory and oxidant-regulated kinase; FARE-1, fatty acid response element 1; RLU, relative light unit(s); DBD, DNA binding domain.

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Abstract
dant stressors (reviewed in Ref. 20). In the intact heart, p38 kinase is activated by pressure overload and, in cultured cardiac myocytes, by hypoxic stimuli, such as α1-adrenergic agonists and cyclic strain (18, 19, 21). The p38 kinase pathway is also activated by cardiac ischemia or hypoxia and has been linked both to the cardiac myocyte apoptotic program and to the protective effects of ischemic preconditioning (19, 22–24). Given the regulation of PPARα activity during cellular stress, the present study sought to examine whether the p38 stress-activated protein kinase signal transduction cascade influences PPARα activity in heart. Herein, we show that PPARα exists as a phosphoprotein in cardiac myocytes and that p38 activation significantly enhances this state of phosphorylation, leading to an increase in ligand-dependent transactivation of targets and enhanced cooperation with the transcriptional coactivator PGC-1.

**EXPERIMENTAL PROCEDURES**

**Primary Rat Neonatal Cardiac Myocyte Cell Culture—**Ventricular cardiac myocytes were isolated from 1–2-day-old rats as described (4) with the following modifications. Myocytes were maintained on dishes pretreated with 0.1% gelatin (Speciality Media). After 24 h in DMEM (4.5 g/liter glucose) supplemented with 10% horse serum, 5% fetal calf serum, bromodeoxyuridine (100 μM), L-glutamine (2 mM), and Fungi-<sup>−</sup>serum, bromodeoxyuridine (100 μM), L-glutamine (2 mM), and Fungi-<sup>−</sup>medium was changed to serum-free DMEM (4.5 g/liter glucose) supplemented with bromodeoxyuridine, L-glutamine, Fungizone, transferin (10 μg/mL), insulin (10 ng/mL), and essentially fatty acid-free BSA (1 mg/mL). Ligands, agonists, and inhibitors were added to the medium after an additional 12 h as described below.

**Plasmids and Transient Transfection Studies—**Cardiac myocyte transient transfections were performed using the calcium phosphate method as described (25) with the following modifications: 4 μg of reporter DNA (MCPT.Luc.781 or MCPT.Luc.781.m1; Ref. 13) were used per well in 12-well plates. SB202190 (20 μM) was added 12 h after transfection as indicated. CV-1 transient transfections were performed using the calcium phosphate manufacturer's protocol. CV-1 cells were transfected with 4 μg of expression plasmids per well. (ACO)3TKLuc (4), mP3CR-Luc.781, and (UAS)3TKLuc from David D. Moore (Baylor College of Medicine, Houston, TX). Expression vectors for wild-type p38 MAPK–GAL4DBD fusion expression vector was created by subcloning a cDNA encoding mouse PPARα into pAdTrackCMV. pCDM-PPARα vector was created by subcloning a cDNA encoding mouse PPARα into pAdTrackCMV and obtained along with (UAS)3TKLuc from David D. Moore (Baylor College of Medicine, Houston, TX). Expression vectors for wild-type and mutant fusion proteins were incubated with [γ-<sup>32</sup>P]ATP in the presence (+) or absence (−) of activated p38α MAPK. Partial MAPK recognition sequences are shown at the bottom, including the specific combinations of serine residues mutated to alanine to create GST-PPARα-S6-21A, GST-PPARα-S6-244 and p38α: WT, S6-21A, S73-77A, S6-21A, and p38α: WT, S6-21A, S73-77A, S6-21A. Labeled GST-PPARα was detected via Western blotting with anti-PPARα antisera.

**In Vitro Kinase Studies—**A murine PPARα cDNA containing an NH<sub>2</sub>-terminal FLAG epitope was cloned in-frame with GST in pGEX-4T-1 (Amersham Pharmacia Biotech). Recombinant protein was expressed in BL21 bacteria and partially purified according to the manufacturer’s protocol. GST-PPARα protein was left bound to glutathione-Sepharose 4B, and in vitro kinase assays were performed with activated p38α kinase (Upstate Biotechnology, Inc.) in 1× kinase reaction buffer (Stratagene). Reactions were allowed to proceed for 30 min at 30°C, and products were subjected to SDS-PAGE. Site-directed mutagenesis of pGEX-PPARα was performed as above using the same oligonucleotides to produce recombinant proteins containing the identical mutations present in the PPARα-GAL4DBD plasmid series.

**Statistical Analysis—**Values presented in graphs are mean ± standard error of the mean (S.E.). Differences between values were analyzed by a one-factor analysis of variance or unpaired Student’s t test.

**RESULTS**

**Activation of SAPK Pathways Leads to Phosphorylation of PPARα in Cardiac Myocytes—**To determine whether PPARα is a target for SAPK-mediated phosphorylation in cardiac myocytes, 32P labeling of nonstimulated, epitope-tagged PPARα in primary cultures of neonatal rat cardiac myocytes was performed under serum-free conditions. Immunoprecipitation of 32P-labeled FLAG-PPARα demonstrated that PPARα exists as a phosphoprotein under basal culture conditions in cardiac myocytes (Fig. 1, lane 1). To determine whether SAPK pathways contribute to the phosphorylation of PPARα in myocardial cells, we performed experiments as described (29). Cardiac myocytes were infected with FLAG-PPARα-expressing adenovirus 24 h after initial plating at a multiplicity of infection sufficient to infect greater than 95% of the cells based on green fluorescent protein fluorescence. Some plates were treated with SB202190 for 48 h prior to orthophosphate labeling. The cells were then washed and maintained in phosphate-free DMEM supplemented with 1 μCi of H<sub>3</sub>P<sub>31</sub>O<sub>4</sub> for 3 h. At that time, plates were treated with anisomycin (0.2 μg/mL) (Calbiochem) or MeSO<sub>4</sub> vehicle with and without SB202190 for an additional 30 min. Following labeling, the cells were washed and scraped into phosphate-buffered saline supplemented with 1× Complete protease inhibitor mixture (Roche Molecular Biochemicals), Na<sub>2</sub>VO<sub>4</sub> (200 μM), Na<sub>3</sub>P<sub>2</sub>O<sub>7</sub> (100 μM), and phenylmethylsulfonyl fluoride (0.1 mg/mL). Cells were pelleted and lysed in RIPA buffer plus protease and phosphatase inhibitors as above. Lysates were precleared with Protein L-Sepharose (Pierce) and then incubated with anti-FLAG M2 antiserum (Sigma) overnight at 4°C. Immune complexes were collected on Protein L-Sepharose and electrophoresed via SDS-PAGE. The proteins were transferred to nitrocellulose and imaged via phosphorimager. Western blot analysis to demonstrate loading was performed with anti-PPARα antibody (provided by John Woods and Joel Berger, Merck Co.).
cytes, phospholabeling experiments were performed in the presence of SB202190, an inhibitor primarily of the p38 kinase pathway. The presence of SB202190 reduced the phosphorylation of FLAG-PPARα (Fig. 1, lane 2). Conversely, a brief exposure to anisomycin, an activator of both p38 and JNK kinases, the major SAPK pathways in cardiac myocytes, dramatically increased levels of phosphorylated PPARα (Fig. 1, lane 3). Finally, SB202190 prevented the anisomycin-induced increase in PPARα phosphorylation (Fig. 1, lane 4). Given that SB202190 is capable of inhibiting p38 MAPK and, under certain conditions, the JNK pathway, these data are consistent with PPARα serving as a downstream target of either the p38 MAPK or JNK pathway in cardiac myocytes.

p38 MAPK Directly Phosphorylates PPARα—The phosphorylation of PPARα shown above could occur as a result either of direct phosphorylation by SAPKs or phosphorylation via other downstream kinases. To examine whether PPARα is a direct substrate of p38 kinase, in vitro kinase assays were performed. Incubation of GST-PPARα fusion proteins with activated p38α kinase resulted in a robust phosphorylation of PPARα (Fig. 2). Examination of the primary amino acid sequence of murine PPARα reveals a number of putative MAPK (S/T)P recognition sequences, all of which are located in the NH2-terminal A/B domain (Fig. 2). To localize the primary phosphoacceptor sites within PPARα, the in vitro kinase assay was repeated with mutant PPARα proteins containing substitutions of nonphosphorylatable alanines for serines at amino acid positions 6/12/21 (S6—21A), 73/76/77 (S73—77A), or all six putative phospho-acceptor serines (S6—77A). Examination of the relative degree of phosphorylation of the mutant PPARα proteins demonstrated that the major serine phosphorylation sites are localized within the grouping of serines at positions 6, 12, and 21. Although S6—21A is still phosphorylated, this occurs at a significantly reduced degree relative to wild-type and the S73—77A mutant. As expected, no phosphorylation of the S6—77A mutant was observed in this assay, effectively localizing all the p38 kinase phospho-acceptor sites to within the A/B domain.

p38 MAPK Activity Is Necessary for Full PPARα Transactivating Function in Cardiac Myocytes—To determine whether activated p38 kinase affects PPARα transactivating function in cardiac myocytes, transient transfection studies were performed with a luciferase reporter construct (MCPT.Luc.781) containing the promoter from the human muscle-type carnitine palmitoyltransferase I (M-CPT I or CPT Iα) gene, a known cardiac PPARα target involved in the mitochondrial FAO pathway (13). MCPT.Luc.781 was transiently transfected into rat neonatal cardiac myocytes in the absence and presence of SB202190. M-CPT I promoter activity was reduced greater than 70% by addition of SB202190 to the medium (Fig. 3). When a M-CPT I promoter-reporter construct containing a mutated PPAR response element (MCPT.Luc.781.m1, Ref. 13) was used in identical experiments, p38 kinase inhibition had no effect, indicating that an intact PPAR binding site is necessary for the p38 MAPK effect (Fig. 3). These results together with the in vitro kinase data suggest that phosphorylation by p38 kinase augments PPARα-mediated activation of M-CPT I gene transcription in cardiac myocytes.

To further examine the functional interaction between p38 kinase and PPARα, MCPT.Luc.781 transfections were performed in CV-1 cells, which are functionally null for PPARα, RXR, and activated p38 kinase. Activation of p38 MAPK was achieved by cotransfection with a constitutively active upstream kinase of p38 kinase (MKK6b/E) and wild-type p38α. Fig. 4A shows that neither p38 kinase activation, via cotransfection of MKK6b/E with p38α, nor treatment with SB202190 affected the basal activity of MCPT.Luc.781 in CV-1 cells. Ligand-mediated activation of cotransfected PPARα and RXRα was demonstrated with the addition of oleic acid, a known PPARα ligand, to the culture medium (Fig. 4A). In the presence of activated p38 MAPK, the ligand-mediated PPARα induction of M-CPT I promoter activity was significantly greater relative to treatment with ligand alone (20-fold versus 6-fold; Fig. 4A). This p38 kinase mediated-enhancement of PPARα activity was inhibited by SB202190, confirming that the effect was specific for the p38 kinase pathway. Thus, activated p38 kinase significantly enhances the transactivation properties of the PPARα/RXRα heterodimer.

To exclude the possibility that the p38 kinase effects are mediated by PPAR-independent pathways via elements other than the PPARα response element (FARE-1) within the M-CPT I promoter, the cotransfection experiments were repeated with a reporter containing an independent PPAR response element derived from the peroxisomal acyl-CoA oxidase (ACO) gene, upstream of a heterologous promoter (A(CoA)TKLuc) (Fig. 4B). As was observed with MCPT.Luc.781, PPARα/RXRα-mediated activation was significantly reduced in the presence of SB202190, confirming that the effect was specific for the PPARα/RXRα heterodimer. The reporter constructs are shown schematically at the top, including the sequence of the wild-type and mutated PPAR response element, FARE-1. The bars represent mean luciferase activity (in relative luciferase units or RLU ± S.E.) normalized (1.0) to the activity of MCPT.Luc.781 under basal culture conditions. The data represent the mean of at least three independent experiments. The asterisk (*) denotes a significant difference (p < 0.05) between the indicated conditions.
transactivation of (ACO)₃TKLuc was significantly increased by cotransfection of p38 kinase and MKK6b(E) (Fig. 4B). In this series of experiments, MKK6b(E)/p38α cotransfection activated PPARα/RXRα heterodimers both in the absence and presence of exogenous ligand.

To confirm that PPARα rather than its heterodimeric partner RXR was the direct functional target of activated p38 kinase in the transfection experiments described above, a modified mammalian one-hybrid system was employed. For these experiments, a full-length PPARα-GAL4 DNA-binding domain fusion protein (PPARα-GAL4DBD) was cotransfected with a GAL4-responsive reporter ((UAS)₃TKLuc) and MKK6b(E)/p38α. The PPARα-GAL4DBD fusion protein retains the ability to be activated by PPARα ligand to a similar degree as that observed earlier in the PPARα/RXRα heterodimer transfections (Fig. 5). Cotransfection of MKK6b(E)/p38α with PPARα-GAL4DBD revealed that, in the absence of PPARα ligand, p38α kinase does not activate PPARα-GAL4DBD (Fig. 5). However, a significant increase in PPARα-GAL4DBD activity is seen with p38α activation in the presence of PPARα ligand, demonstrating a ligand-mediated induction of ~20-fold in the presence of p38 MAPK activation versus 6-fold ligand-mediated induction in the absence of p38 MAPK activation. To exclude the possibility that these results were caused by spurious activation of the JNK pathway, the (UAS)₃TKLuc cotransfections were repeated with an expression vector for a c-Jun-GAL4DBD hybrid protein, a known JNK-specific target. c-Jun-GAL4DBD was not activated by MKK6b(E)/p38α but was increased (5-fold) by cotransfection of JNK and its activator MEKK (data not shown), indicating that the observed activation of PPARα-GAL4DBD in CV-1 cells was the result of the specific effects of the p38 kinase pathway. To confirm that the PPARα-GAL4DBD fusion protein does not heterodimerize with endogenous RXR in CV-1 cells, parallel control experiments were performed with the RXR ligand, 9-cis-retinoic acid, in the presence or absence of cotransfected RXRα. Addition of 9-cis-retinoic acid with or without cotransfection of RXRα had no effect on the target reporter activity in the presence of PPARα-GAL4DBD (data not shown), confirming that RXRα is not interacting with PPARα-GAL4DBD in this system and, therefore, is not the mediator of the effect of p38 kinase on PPARα/RXRα heterodimer transactivation. These results indicate that p38
MAPK activates PPARα in a RXR-independent manner. Moreover, these data demonstrate that the activation of PPARα by p38 kinase is independent of effects on DNA binding.

**p38 MAPK-mediated Activation of PPARα Maps to Phosphorylation Sites within the A/B Domain**—To determine whether the phosphorylation sites identified within the PPARα A/B domain in *in vitro* kinase studies confer the functional effects shown above, the mammalian one-hybrid experiments were repeated using full-length PPARα-GAL4DBD expression vectors containing the same mutations used for the *in vitro* kinase assays. Fig. 6A shows that, as predicted by the results of the *in vitro* kinase assays, the S6–21A and S6–77A mutants are not responsive to p38α kinase in the absence or presence of PPARα ligand (Fig. 6A and data not shown). However, MKK6b(E)/p38α-mediated activation of the S73–77A fusion protein is similar to the wild-type protein (Fig. 6A), a result that is also consistent with the results of the *in vitro* phosphorylation studies. Fig. 6B shows that the mutant PPARα-GAL4DBD fusion proteins retain the ability to be activated by PPARα ligand, indicating that A/B domain phosphorylation is not necessary for ligand-dependent AF-2 function in CV-1 cells. These data indicate that phosphorylation of PPARα by p38 kinase on one or more of the serines at position 6, 12, or 21 is responsible for p38-mediated enhancement of PPARα transactivation function.

**Activated p38 MAPK Enhances Coactivation of PPARα by PGC-1**—Ligand activation of nuclear receptors leads to recruitment of transcriptional coactivators. We sought to test whether the activation of PPARα by p38 MAPK phosphorylation involved the action of specific coactivators. Cotransfection experiments were performed with PPARα-GAL4DBD expression vectors for wild-type or mutant PPARαs and known PPARα coactivators, including PGC-1 (28), SRC-1 (30), and PBP (31). As we have shown previously (28), the wild-type PPARα-GAL4DBD fusion protein was activated by PGC-1 in the presence of ligand (Fig. 7). When cotransfection of PGC-1 was combined with MKK6b(E)/p38α in the presence of ligand, a dramatic increase in PPARα activation was seen, nearly 6-fold relative to PGC-1 cotransfection in the absence of p38 MAPK activation (Fig. 7). In striking contrast, although the PPARαs-S6–21A-GAL4DBD mutant was PGC-1-responsive to the same degree as wild-type PPARα-GAL4DBD, it was not activated further by MKK6b(E)/p38α in the presence or absence of ligand (Fig. 7). Unlike the results with PGC-1, neither SRC-1 nor PBP coactivation of PPARα-GAL4DBD was influenced by activation of p38 MAPK in these experiments (data not shown), suggesting that phosphorylation of PPARα serves to enhance coactivation by a specific subset of coactivators.

**DISCUSSION**

PPARα, a lipid-activated transcription factor, plays a critical role in the control of cellular energy metabolism in a variety of physiologic and pathologic states. Evidence has emerged that PPARα activity is modulated in heart and liver during diverse stress responses, including fasting (1), cardiac hypertrophy (4), and cellular hypoxia (32). This implies that upstream signaling events activated by cellular stressors are linked to changes in PPARα activity, which in turn regulates mitochondrial energy metabolism. Members of the p38 kinase family, so-called “stress-activated protein kinases,” represent likely candidates to serve as upstream regulators of PPARα. In this report, we show that p38 kinase-mediated phosphorylation activates PPARα in a ligand-influenced manner and results in enhanced functional cooperation with the transcriptional coactivator PGC-1. These results suggest that p38 kinase signaling promotes cardiac mitochondrial fatty acid β-oxidation during periods of stress.

Certain pathologic conditions lead to a decrease in myocardial oxidative energy production through reduced FAO enzyme gene expression linked to antagonism of PPARα activity. For example, the PPARα gene regulatory pathway is deactivated during α1-adrenergic agonist stimulation of cardiac myocyte hypertrophy (4). We have shown that PPARα activity is diminished by a post-transcriptional mechanism mediated by ERK-MAPK, confirming that signal transduction cascades linked to G-protein-coupled receptors can affect the activity of PPARα. Similarly, the related nuclear receptor, PPARγ, is deactivated by ERK-mediated phosphorylation through a mechanism that reduces affinity for ligand (33–36). Given these previous findings, the results shown here indicating that phosphorylation of PPARα by p38 MAPK leads to activation of PPARα function was surprising. Taken together with the results of the ERK-MAPK studies (4), we conclude that distinct limbs of the MAPK network, namely ERK and p38, have opposing effects with respect to PPARα activity in the heart. The molecular mechanism(s) underlying this differential response of PPARα to MAPK signaling remains unknown. Our results do not exclude the possibility that, in certain cellular contexts, including cardiac myocytes, the JNK pathway may also alter PPARα activity.
A diverse array of molecular consequences have been attributed to nuclear receptor phosphorylation, including increased or decreased ligand-dependent and ligand-independent activation (reviewed in Ref. 37), enhanced recruitment of cofactors (38–40), reduced affinity for ligand (33), increased or decreased capacity for DNA binding (reviewed in Ref. 37), enhanced or inhibited heterodimerization (41, 42), and susceptibility to proteosomal degradation (43). Our results indicate that p38 inhibited heterodimerization (41, 42), and susceptibility to proteosomal degradation (43). This is similar to the mechanism by which phosphorylation of SF-1 in the AF-1 domain enhances cofactor recruitment only when the ligand-binding domain is present, although SF-1 is not known to be ligand-activated (39). Alternatively, phosphorylation of the AF-1 region may lead to direct recruitment of coactivators to AF-1 as is seen with ERβ (40). However, there is no evidence that PGC-1 interacts with the A/B domain of PPARα, although a separate PPARα/PGC-1 interacting protein could serve as an adaptor.

Our results suggest an alternative mechanism. The earlier studies reported activation of AF-1 activity using a PPARα A/B domain-GAL4DBD fusion construct as the target (47). However, our results demonstrate that the full-length PPARα-GAL4DBD fusion protein has no constitutive (ligand-independent) AF-1 activity in CV-1 cells (Fig. 5). Moreover, p38 MAPK-mediated phosphorylation does not activate PPARα-GAL4DBD in the absence of ligand, indicating that AF-1 function per se within the context of the full-length molecule, is not enhanced by A/B domain phosphorylation. These results suggest a functional, if not physical interaction between the AF-1 and AF-2 regions of PPARα following phosphorylation in the context of engaged ligand. It is possible that AF-1 activity is increased by A/B domain phosphorylation only when PPARα is ligand-bound, leading to enhanced interaction with specific coactivators, such as PGC-1. This is similar to the mechanism by which phosphorylation of SF-1 in the AF-1 domain enhances cofactor recruitment only when the ligand-binding domain is present, although SF-1 is not known to be ligand-activated (39). Alternatively, phosphorylation of the AF-1 region may lead to direct recruitment of coactivators to AF-1 as is seen with ERβ (40). However, there is no evidence that PGC-1 interacts with the A/B domain of PPARα, although a separate PPARα/PGC-1 interacting protein could serve as an adaptor.
Fig. 7. p38 MAPK enhances coactivation of PPARα by PGC-1. (UAS)TKLuc and PPARα-GAL4DBD wild-type (solid bars) or S6–21A mutant (hatched bars) were co-transfected into CV-1 cells with or without expression vectors for PGC-1, MKK6b(E), and p38α. Cells were maintained in media supplemented with oleic acid (250 μM) or vehicle control (BSA). The bars represent mean RLUs normalized (=1.0) to the activity of (UAS)TKLuc in the presence of PPARα-GAL4DBD wild-type (solid bars) or S6–21A mutant (hatched bars) under basal culture conditions. The data represent the mean of at least three independent experiments. The asterisk (*) indicates a significant difference (p < 0.05) between the indicated conditions.

In summary, we have shown that p38 MAPK phosphorylates and activates the transcription factor PPARα, leading to enhanced ligand-mediated coactivation by the transcriptional co-activator PGC-1. These results identify PPARα as a target of stress-activated signaling. In cardiac myocytes, p38 MAPK activation was predicted to increase the capacity for energy production by the mitochondrial fatty acid β-oxidation pathway, as a component of the metabolic response to diverse physiologic stressors.

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