Potential of liver X receptor transcriptional activity by peroxisome-proliferator-activated receptor γ co-activator 1 α

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Peroxisome-proliferator-activated receptor (PPAR) γ co-activator 1 α (PGC-1 α /PPARGC1) plays an important role in energy metabolism by co-ordinating transcriptional programmes of mitochondrial biogenesis, adaptive thermogenesis and fatty acid β -oxidation. PGC-1 α has also been identified to play a role in the intermediary metabolism by co-activating key transcription factors of hepatic gluconeogenesis and glucose uptake in muscles. In the present study, we show that PGC-1 α serves as a co-activator for the liver X receptor (LXR) α , known to contribute to the regulation of cellular cholesterol homeostasis. In transient transfection studies, PGC-1 α amplified the LXR-mediated autoregulation of the LXR α promoter in a human brown adipocyte line and in 3T3-L1 cells via an LXR response element described previously. LXR-mediated transactivation via a natural LXR response element from the cholesterol ester transfer-protein gene promoter was also enhanced by PGC-1 α in a ligand-dependent manner. Mutational analysis showed that the

LXXLL signature motif (L2) of PGC-1 α was essential for co-activation of LXR-mediated transcriptional responses. This motif is located in the vicinity of the binding region for a putative repressor described previously. The repressor sequesters PGC-1 α from PPAR α and the glucocorticoid receptor, and this repressor did not interfere with PGC-1 α -mediated co-activation of LXR-dependent gene transcription. Moreover, inhibition of p38 mitogen-activated protein kinase signalling, shown to abolish the co-activation of PPAR α by PGC-1 α , had only a moderate inhibitory effect on the co-activation of LXR. These results identify PGC-1 α as a *bona fide* LXR co-activator and implicate distinct interfaces of PGC-1 α and/or additional cofactors in the modulation of LXR and PPAR α transcriptional activities.

Key words: liver X receptor response element, nuclear hormone receptor, p38 mitogen-activated protein kinase, transcriptional co-activator.

INTRODUCTION

Liver X receptor (LXR) α (NR1H3) [1] and LXR β (NR1H2) [2,3], originally identified as orphan receptors, were adopted as nuclear hormone receptors when oxysterols were recognized as their natural ligands [4,5]. LXR α is expressed mainly in liver, intestine, adipose tissue and macrophages [1], whereas LXR β is expressed in many tissues [2,3]. Both receptor subtypes form obligate heterodimers with the retinoid X receptor (RXR). The LXR–RXR heterodimers bind preferentially to hormone response elements [termed LXR response elements (LXREs)], which consist of two direct hexanucleotide repeats separated by four nucleotides (DR4) [1]. LXR α is involved in the transcriptional control of a number of genes regulating the transport and catabolism of cholesterol [4,5] and synthesis of unsaturated fatty acids [6,7]. Identified LXR α target genes include CYP7A1, encoding cholesterol 7 α -hydroxylase, the rate-limiting enzyme in neutral bile acid synthesis [8], the ATP-binding cassette transporter (ABC) A1 [9], apolipoprotein E [10] and cholesterol ester transfer protein (CETP) [11], involved in reverse cholesterol transport, ABCG1 [12], ABCG4 [13], ABCG5 and ABCG8 [14], concerned in intracellular trafficking of sterols [15], and the sterol-regulatory element-binding protein 1c, engaged in the dietary regulation of hepatic lipogenic enzymes [16,17]. Thus LXR α may integrate a transcriptional programme that protects

cells against accumulation of free cholesterol. The biological roles of LXR β remain unclear, although they are likely to be distinct [18].

Peroxisome-proliferator-activated receptor (PPAR) γ co-activator 1 α (PGC-1 α /PPARGC1) [19–21], PGC-1 β [22] and the PGC-1-related protein [23] belong to a recently discovered family of transcriptional co-activators that share similar tissue distributions. PGC-1 α was originally described as a cold-inducible co-activator that serves as the master regulator of transcriptional programmes for mitochondrial biogenesis, respiration and thermogenesis [19,24]. Several lines of evidence suggest that PGC-1 α also plays an important role in intermediary metabolism. PGC-1 α co-activates MADS box transcription factor 2C, thereby controlling endogenous GLUT4 mRNA expression in the muscle [25]. Moreover, studies on cultured hepatocytes and transgenic mice showed that PGC-1 α induces the expression of key enzymes of gluconeogenesis, thereby increasing glucose output by the liver [26,27]. Since intracellular glucose and lipid metabolism are intimately linked and since PGC-1 α co-activates several nuclear hormone receptors that form obligate heterodimers with RXR, we tested the hypothesis that transactivation of LXRs is co-activated by PGC-1 α . We used two natural LXREs from the LXR α and CETP gene promoters to demonstrate that PGC-1 α co-activates LXR in a ligand-dependent manner. An intact L2 motif of PGC-1 α was required for LXR-mediated co-activation.

Abbreviations used: ABC, ATP-binding cassette transporter; CETP, cholesterol ester transfer protein; 9cRA, 9-*cis*-retinoic acid; EMSA, electrophoretic mobility-shift assay; GR, glucocorticoid receptor; 22(R)HC, 22(R)-hydroxycholesterol; LXR, liver X receptor; LXRE, LXR response element; MAPK, mitogen-activated protein kinase; PGC-1 α , PPAR γ co-activator 1 α ; PPAR, peroxisome-proliferator-activated receptor; RXR, retinoid X receptor; TK, thymidine kinase; UCP, uncoupling protein.

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However, a putative PGC-1 α repressor and inhibition of p38 mitogen-activated protein kinase (MAPK) signalling, both of which affect PGC-1 α recruitment by other nuclear hormone receptors [28,29], had either no effect or only a moderate effect on LXR co-activation.

MATERIALS AND METHODS

Materials

DNA restriction and modification enzymes were obtained from New England Biolabs (Beverly, MA, U.S.A.) and Promega (Madison, WI, U.S.A.). Cell culture media, foetal calf serum and LipofectAMINE 2000 were obtained from Life Technologies (Groningen, The Netherlands). 22(R)-hydroxycholesterol [22(R)HC], 9-*cis*-retinoic acid (9cRA), dexamethasone, insulin and tri-iodothyronine were purchased from Sigma (St. Louis, MO, U.S.A.). SB 202190 was obtained from Calbiochem (San Diego, CA, U.S.A.). The Dual-Luciferase Assay System was purchased from Promega.

Plasmid constructs

The LXR α -Prom-Luc plasmid in which the region from -4356 to +209 of the human LXR α gene drives the promoterless firefly luciferase gene was generated using 5'-TCTACGCGTTCTCA-TCTGGTTCCTTCTGTCC-3' (-4356 to -4335) and 5'-TCTCTCGAGGCACGACTAAGAATGACTCTGG-3' (+188 to +209) as upper and lower primers respectively. The amplified 4565 bp DNA fragment was cloned into the pGL3-Basic Vector (Promega). Numbers in parentheses refer to primer positions relative to the transcriptional start site (GenBank® Nucleotide Sequence Database accession no. U22662). *Mlu*I and *Xho*I restriction enzyme sites introduced into the primer sequences are in boldface.

For construction of Δ LXRE-C-Luc harbouring a 5'-terminal deletion of a previously identified LXRE [30,31], a 4197 bp fragment spanning nucleotides -3987 to +209 was amplified using 5'-TCTACGCGTTGGACTCTGACACAGCAAAGG-3' (-3987 to -3967) as upper primer, and the lower primer was as described above. The *Mlu*I restriction enzyme site introduced into the upper primer sequence is in boldface. The resulting fragment was ligated into the pGL3-Basic Vector.

The thymidine kinase (TK)-Luc promoter vector was constructed by ligating the *Bgl*II-*Hind*III fragment derived from the pRL-TK vector (Promega) and encompassing the herpes simplex virus minimal TK promoter region into the pGL3-Basic Vector upstream of the luciferase reporter gene. For generation of the LXRE-C-TK-Luc construct, a 402 bp fragment containing the LXRE-C response element was amplified using 5'-TCTACGCGT-TCTCATCTGGTTCCTTCTGTCC-3' (-4356 to -4335) and 5'-TCTCTCGAGCCTCACTCCAGTCCCTTTGC-3' (-3974 to -3955) as forward and reverse primers respectively, and ligated into the TK-Luc promoter vector. *Mlu*I and *Xho*I restriction enzyme sites introduced into the primer sequences are in boldface. For construction of the 3 $\times$ DR4-TK-Luc plasmid, oligonucleotides 5'-CGCGTGCTTGGGGTCATTGTCGGG-CACAAGCTTGGGGTCATTGTCGGGCACAAGCTTGG-GGTCAATTGTCGGGCAACAGT-3' and 5'-TCGAGACTG-TTGCCCGACAATGACCCCAAGCTTGTGCCCCGACAAT-GACCCCAAGCTTGTGCCCCGACAATGACCCCAAGCA-3' were annealed and ligated into the TK-Luc promoter vector.

A mammalian expression vector containing the human cDNA for LXR α driven by the cytomegalovirus (CMV) immediate-early promoter (LXR α) was generated by reverse transcriptase-

PCR amplification of the entire LXR α coding region (GenBank® accession no. U22662). A 1347 bp cDNA fragment was amplified from human liver total RNA using 5'-TGGGTACCAAAG-AGATGTCCTTGTGGCTGG-3' (+29 to +50) and 5'-TGC-TCGAGTTCGTGGACATCCCAGATCTCA-3' (+1355 to +1376) as forward and reverse primers respectively, and ligated into the pcDNA6/V5-HisA vector (Invitrogen, Leek, The Netherlands). For construction of an LXR β expression vector a 1398 bp cDNA fragment containing the LXR β coding region (GenBank® accession no. NM007121) was amplified by reverse transcriptase-PCR starting from liver total RNA using 5'-TGGGTACCCCCACCATGTCCTCTCTCTACC-3' (+240 to +260) and 5'-TGCTCGAGAGCCCCTAACTCGTGGACGT-3' (+1617 to +1636) as forward and reverse primers respectively, and ligated into the pcDNA6/V5-HisA vector. *Xho*I and *Kpn*I restriction enzyme sites introduced into the primer sequences are in boldface. The PGC-1 α , L2mutPGC-1 α and Δ PGC-1 α -L2mut constructs were described previously [29]. All constructs were verified by dye-terminator cycle sequencing using the ABI Prism™ 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, U.S.A.).

Cell culture, transfections and reporter gene assays

PAZ6 cells were cultured and differentiated as described previously [29,32]. Before transfection, cells were washed twice with Hanks balanced salt solution (Sigma) and incubated for 3 h in preadipocyte medium without drugs. Drugs at the concentration of 10 μ M for 22(R)HC and 9cRA were added immediately before transfection. SB 202190 (20 μ M) was added 2 h before transfection. 3T3-L1 preadipocytes were maintained in Dulbecco's modified Eagle's medium and 10% calf serum. At confluence, the medium was changed to Dulbecco's modified Eagle's medium supplemented with 1.7 μ M insulin, 0.5 mM 3-isobutyl-1-methylxanthine and 1 μ M dexamethasone for 48 h.

Differentiated PAZ6 and 3T3-L1 cells cultured in 24-well dishes were transfected using LipofectAMINE 2000 reagent. Unless otherwise indicated, we used 1 μ g of reporter plasmids, 0.5 μ g of expression plasmids and 20 ng of pRL-TK plasmid (Promega) as transfection control. For transfection of the LXRE-C-TK-Luc and the 3 $\times$ DR4-TK-Luc reporter constructs, 1 ng of pRL-CMV plasmid was used as internal control. Transfected differentiated cells were collected 24 h after transfection and firefly and *renilla* luciferase activities were measured in a luminometer (Anthos Labtec Instruments, Salzburg, Austria) using the Dual-Luciferase Reporter Assay System (Promega). Graphs are representative for 1 of 2 experiments, each performed in quadruplicate. Results are given as means $\pm$ S.D. ANOVA and Tukey's honest significant difference test were used to compare results of transactivation assays.

Electrophoretic mobility-shift assays (EMSAs)

Nuclear extracts from differentiated PAZ6 cells were prepared, and EMSAs were performed as described previously [33]. Wild-type competitor oligonucleotides were added at 30-, 100- and 200-fold molar excess, and mutant oligonucleotides at 100-fold molar excess. Sense strand sequences of oligonucleotides LXRE-C and mutLXRE-C were 5'-aattcGGCAGGGGGTGA-ATGACCAGCAGTAACCTCAGCAGCTTGCC-3' and 5'-aattcGGCAGGGGGTGAATGTTTCAGCAGTAATTCAGCA-GCTTGCC-3' respectively. Overhangs are represented by lower-case letters and mutated nucleotides are underlined.

RESULTS

PGC-1 α potentiates LXR-mediated transcriptional activation of the human LXR α promoter

Autoregulation of the human, but not the murine, LXR α gene promoter has been demonstrated in macrophages, preadipocytes and HepG2 cells [30,31]. To ascertain a possible co-activator function of human PGC-1 α in the LXR-dependent transactivation of the human LXR α gene, we performed co-transfection studies in PAZ6 cells immortalized from human brown adipocytes. A plasmid construct (Figure 1A) containing approx. 4.5 kb of the human LXR α promoter region fused to a luciferase reporter gene (LXR α -Prom-Luc) was transiently transfected into differentiated PAZ6 cells. In agreement with previous studies [30,31], basal transcriptional activity of the LXR α -Prom-Luc reporter gene was stimulated 2-fold ($P < 0.001$) by the LXR-specific ligand 22(R)HC. Only an insignificant additive effect of the RXR agonist 9cRA was observed. Co-transfection with a human PGC-1 α expression plasmid (PGC-1 α) resulted in a 6-fold activation of the reporter gene expression in the presence of 22(R)HC and 30-fold activation in the presence of both 22(R)HC

and 9cRA (Figure 1B). These results indicated that PGC-1 α acted as a potent co-activator of LXR and that both LXR and RXR must be liganded to attain maximal LXR-mediated transcriptional activity of the LXR α promoter. Co-transfection of an LXR α expression construct had little or no effect when compared with the respective controls, suggesting that endogenous LXR expression levels were not limiting in differentiated PAZ6 cells.

To confirm the role of PGC-1 α in LXR-mediated transcriptional activity in a different cellular environment, we performed co-transfection studies in differentiated 3T3-L1 cells. Basal reporter gene expression was stimulated by 22(R)HC ($P < 0.01$) and stimulation was further enhanced by a combination of 22(R)HC and 9cRA ($P < 0.002$). Co-transfection of the LXR α expression plasmid increased the transcriptional activity of the LXR α -Prom-Luc construct by approx. 2-fold in the absence of exogenous ligands ($P < 0.001$), and an additional increase was observed on stimulation of cells with a combination of 22(R)HC and 9cRA. Again, PGC-1 α co-transfection resulted in a substantial activation of reporter gene expression in comparison with the respective controls (Figure 1C). Co-transfection studies showed that LXR α and LXR β expression constructs increased reporter gene activity and demonstrated that PGC-1 α

Figure 1 PGC-1 α potentiates LXR-mediated autoregulation of the human LXR α gene

(A) Schematic representation of the LXR α -Prom-Luc construct. LXRE-A, -B and -C represent putative LXREs identified previously. PPRE designates a PPAR response element. (B) Differentiated PAZ6 cells were transiently transfected with LXR α -Prom-Luc or co-transfected with a human PGC-1 α expression construct (PGC-1 α) or a human LXR α expression construct (LXR α). Activity levels of LXR α -driven firefly luciferase were standardized to pRL-TK-driven *renilla* luciferase levels, used as transfection control. Cells were incubated with 22(R)HC, 9cRA or both, each at a concentration of 10 μ M. (C) Differentiated 3T3-L1 cells were used in transient transfection experiments as described above. (D) Differentiated 3T3-L1 cells incubated with 22(R)HC and 9cRA were transfected with LXR α -Prom-Luc or co-transfected with PGC-1 α and/or LXR α and LXR β expression constructs as indicated. Fold induction refers to basal LXR α -Prom-Luc reporter-gene expression levels in the absence of PGC-1 α , LXR α and LXR β expression plasmids or drugs.

Figure 2 The LXRE-C-binding site is essential for PGC-1 α -mediated transcriptional co-activation of the human LXR α gene

Schematic representation of (A) the Δ LXRE-C-Luc construct and (B) the LXRE-C-TK-Luc construct. (C) Differentiated PAZ6 cells were transfected with Δ LXRE-C-Luc or co-transfected with LXR α or PGC-1 α in the presence or absence of 22(R)HC and/or 9cRA. (D) Differentiated PAZ6 cells were transfected with LXRE-C-TK-Luc or co-transfected with LXR α or PGC-1 α in the presence or absence of 22(R)HC and/or 9cRA. Fold induction refers to basal Δ LXRE-C-Luc or LXRE-C-TK-Luc expression levels in the absence of expression plasmids and drugs.

enhanced both LXR α and LXR β transcriptional activities in the presence of 22(R)HC and 9cRA (Figure 1D).

PGC-1 α co-activates the human LXR α promoter via a putative LXRE in differentiated human brown adipocytes

Of three putative LXREs, the most distal site, termed LXRE-C, has been identified as the primary element that mediated induction of the LXR α promoter by LXR α /RXR α heterodimers [30,31]. To determine whether LXRE-C is the site associated with PGC-1 α co-activation, co-transfection studies were performed with PGC-1 α and Δ LXRE-C-Luc carrying a 5'-terminal deletion of LXRE-C (Figure 2A). In comparison with co-transfection studies of LXR α -Prom-Luc (Figure 1B), transcriptional activity of the Δ LXRE-C-Luc reporter construct was decreased to basal levels in cells co-transfected with PGC-1 α and stimulated with 22(R)HC alone or in combination with 9cRA (Figure 2C). To corroborate these results, we cloned LXRE-C upstream of a minimal TK promoter linked to the firefly luciferase gene (Figure 2B). 22(R)HC treatment of differentiated PAZ6 cells transiently transfected with LXRE-C-TK-Luc resulted in a 1.9-fold stimulation of reporter gene activity. No additive effect was observed on co-stimulation with 9cRA. Co-transfection with LXR α resulted in a slight increase in reporter gene activity in the absence ($P < 0.003$) or presence of ligands [$P < 0.05$ for 22(R)HC or $P < 0.005$ for 22(R)HC and 9cRA]. Co-transfection with PGC-1 α strongly potentiated LXRE-C-mediated transcriptional activity in a ligand-dependent manner (Figure 2D). These results

indicated that LXRE-C is the response element that is involved in the LXR-dependent activation and PGC-1 α -mediated co-activation of the human LXR α promoter.

Specific binding of nuclear proteins derived from differentiated PAZ6 cells to an LXRE-C probe was shown in bandshift assays. Increasing amounts of unlabelled wild-type DNA effectively competed with the labelled probe for binding of nuclear proteins, whereas a double-stranded oligonucleotide harbouring a mutated LXRE-C sequence failed to compete (Figure 3). These results are consistent with our transfection studies showing that LXRE-C is the main element mediating autoregulation of the LXR α gene.

To ascertain further the co-activator function of PGC-1 α for LXR-dependent transactivation of other LXR target genes, transient transfection studies were performed using a direct-repeat DR4 element contained within the human CETP gene promoter [11]. We constructed a 3 $\times$ DR4-TK-Luc reporter plasmid containing three copies of the DR4 response element upstream of a minimal TK promoter (Figure 4A). Co-transfection studies of 3 $\times$ DR4-TK-Luc with LXR α or PGC-1 α in differentiated PAZ6 cells revealed an induction pattern (Figure 4B) that was comparable with the pattern observed in transfections with the LXRE-C-TK-Luc construct (Figure 2D).

An intact LXXLL motif in PGC-1 α is essential for co-activation of LXR transcriptional activity

The LXXLL motif (L2 at amino acids 145–149) in PGC-1 α has been shown to be essential for the interaction of PGC-1 α with the

Figure 3 EMSAs reveal binding of nuclear protein(s) from differentiated PAZ6 cells to LXRE-C

EMSA were performed using a α -³²P-labelled LXRE-C probe, and nuclear extracts were prepared from differentiated PAZ6 cells. Nuclear extracts were preincubated in the absence (no competitor) or presence of 30-, 100- or 200-fold molar excess of double-stranded unlabelled LXRE-C (LXRE-C) or 100-fold molar excess of double-stranded unlabelled mutated LXRE-C (mutLXRE-C). neg refers to a control reaction without the addition of nuclear extract.

ligand-binding domain of several nuclear hormone receptors, including PPAR α [34], glucocorticoid receptor (GR) [35] and the oestrogen and thyroid hormone receptors [36,37]. To determine whether the L2 motif is essential for co-activation of the LXR α promoter, the double leucine residue was substituted by a double alanine residue (Figure 5A). This mutation has been shown to disrupt leucine-rich interaction motifs [38]. Co-transfections of LXR α -Prom-Luc with the mutated PGC-1 α expression construct (L2mutPGC-1 α) showed that the absence of an intact L2 motif decreased luciferase activity almost to control levels in unstimulated cells and in cells treated with 22(R)HC or 22(R)HC and 9cRA (Figure 5B). Thus the LXXLL motif of PGC-1 α was necessary for co-activation of LXR-mediated transactivation.

A putative repressor of GR- and PPAR α -mediated transcriptional activation does not interfere with PGC-1 α co-activation of LXR-dependent responses

Previous studies showed that the interaction of PGC-1 α with GR and PPAR α is negatively regulated via competitive binding of

Figure 4 PGC-1 α enhances LXR-mediated transcriptional activity via an LXRE from the human CETP gene

(A) Schematic representation of the 3 $\times$ DR4-TK-Luc construct. (B) Differentiated PAZ6 cells were transfected with 3 $\times$ DR4-TK-Luc or co-transfected with LXR α or PGC-1 α in the absence or presence of 22(R)HC or 22(R)HC and 9cRA. Fold induction refers to basal 3 $\times$ DR4-TK-Luc expression levels.

PGC-1 α to a putative repressor [28,29]. Both nuclear receptors interact with PGC-1 α via its L2 domain, whereas the L3 motif (amino acids 211–215) has been identified as the main structural motif involved in repressor binding [28]. Since the L2 region was also essential for amplification of LXR-mediated transactivation, we studied whether the putative repressor competes with LXR for binding to PGC-1 α . We used a decoy molecule described previously [29] to trap the repressor and determined the ability of PGC-1 α to enhance the activity of LXR α -Prom-Luc reporter constructs in the presence or absence of the decoy molecule (Figure 6A). Unlike co-activation of PPAR α , co-activation of LXR by PGC-1 α was not affected by co-transfection of the decoy construct in PAZ6 cells (Figure 6B).

PGC-1 α -mediated co-activation of LXR transcriptional activation is only partially p38 MAPK-dependent

p38 MAPK signalling has been shown to enhance PGC-1 α -mediated co-activation of GR by reducing the interaction of PGC-1 α with its repressor [28,29]. Specific inhibition of p38 MAPK with SB 202190 abolished completely PGC-1 α -mediated co-activation of PPAR α -dependent transactivation of the uncoupling protein (UCP) 1 gene [29]. We therefore studied the effect of SB 202190 on PGC-1 α -mediated potentiation of LXR-dependent transcriptional activation. Co-transfection studies with LXR α -Prom-Luc and PGC-1 α revealed that SB 202190 decreased luciferase activity to basal levels in cells stimulated with 22(R)HC, but much less inhibition was observed on treatment with 22(R)HC and 9cRA (Table 1).

Figure 5 Mutation of the PGC-1 α L2 domain impairs co-activation of LXR by PGC-1 α .

(A) Constructs used in transfection experiments. PGC-1 α , full-length human PGC-1 α (amino acids 1–798); L2mutPGC-1 α , PGC-1 α harbouring two Leu to Ala mutations in the LXXLL motif (L2); AD, activation domain; PPAR γ , PPAR γ -binding domain; L2 and L3, LXXLL motifs. (B) Differentiated PAZ6 cells were transfected with LXR α -Prom-Luc or co-transfected with wild-type PGC-1 α or L2mutPGC-1 α . Reporter gene activity was determined after stimulation of cells with 22(R)HC or 22(R)HC and 9cRA, each at a concentration of 10 μ M. Fold induction refers to basal LXR α -Prom-Luc expression levels.

DISCUSSION

Nuclear receptors are ligand-activated transcription factors that regulate the expression of a wide array of target genes to affect numerous physiological processes, including development and metabolism. Co-activators enhance their transcriptional activity by modifying chromatin structure and/or establishing contact with the transcription machinery. They also play pivotal roles in the promoter- and tissue-specific responses of nuclear receptor action [39]. PGC-1 α was originally identified as a co-activator of PPAR γ [19] and has since been shown to co-activate a number of other nuclear receptors, including PPAR α [34], GR [35], the retinoic acid receptor [29], RXR [40] and the oestrogen [36], thyroid [37] and mineralocorticoid receptors [35]. In the present study, we extend the role of PGC-1 α in nuclear receptor action and demonstrate that it serves as a potent co-activator of LXRs. We show that PGC-1 α amplifies the effects of 22(R)HC and 9cRA on LXR-mediated transcriptional activation of the LXR α gene in two different cell lines. Our results indicate that both LXR α and LXR β are co-activated by PGC-1 α and the DNA region involved maps to an LXRE identified previously in the LXR α promoter. Furthermore, we confirmed the co-activator function of PGC-1 α using a natural LXRE from the human CETP gene [11] and demonstrated that the LXXLL (L2) motif of PGC-1 α is required for the ligand-dependent interaction with LXRs. This α -helical motif, also termed as NR box [38], has been shown to be essential for PGC-1 α -mediated binding and co-activation of GR [35], PPAR α [29,34], RXR [40] as well as the thyroid [37] and oestrogen receptors [36], but is not required for co-activation of PPAR γ [19], the oestrogen-related receptors [41]

Figure 6 Removal of a putative repressor has no effect on PGC-1 α -mediated co-activation of the LXR-dependent transcription of the LXR α gene.

(A) Constructs used in transfection experiments. Δ PGC-1 α -L2mut, decoy construct encompassing amino acids 92–292 and harbouring the mutation in the L2 motif together with deletions of the transcriptional activation domain and the PPAR γ -binding domain. (B) Differentiated PAZ6 cells were co-transfected with LXR α -Prom-Luc and PGC-1 α in the absence or presence of Δ PGC-1 α -L2mut. Transfected cells were incubated without drugs or with 22(R)HC or 22(R)HC and 9cRA, each at a concentration of 10 μ M. Fold induction refers to basal LXR α -Prom-Luc expression levels in cells co-transfected with PGC-1 α .

Table 1 The p38 MAPK inhibitor SB 202190 moderately impairs PGC-1 α -mediated co-activation of LXR-dependent transcription of the LXR α gene

PAZ6 cells co-transfected with LXR α -Prom-Luc and PGC-1 α were stimulated with 22(R)HC or 22(R)HC and 9cRA, each at a concentration of 10 μ M, in the absence or presence of SB 202190 (20 μ M). Data represent fold induction of reporter gene activity $\pm$ S.D., where the luciferase activity of transfected cells without drug stimulation was set as 1.0.

Drug stimulation	Reporter gene expression	
	Without SB 202190	With SB 202190
Basal	1.00 $\pm$ 0.18	1.03 $\pm$ 0.06
22(R)HC	2.12 $\pm$ 0.21*	1.12 $\pm$ 0.24*
22(R)HC + 9cRA	8.14 $\pm$ 0.41†	4.85 $\pm$ 0.39†

* $P < 0.01$.

† $P < 0.001$; ANOVA, Tukey's *post-hoc* comparison.

and other transcription factors, including MADS box transcription factor 2C [25].

Since PGC-1 α participates in a broader spectrum of transcriptional responses than what was initially anticipated, mechanisms are required that confer specificity to interactions with distinct transcription factors. Such mechanisms may operate at several levels of regulation. Distinct protein domains of PGC-1 α are used for interaction with various transcription factors [19,42]. Post-translational modifications of transcription factors and/or co-activators serve as an additional layer that regulates the specificity of interactions. Cytokines have been shown to stabilize and activate PGC-1 α by phosphorylation of three conserved

amino acid residues by p38 MAPK [43]. Although the increased PGC-1 α tissue level resulting from decreased degradation explained some transcriptional responses to cytokine administration, it did not account for the increase in uncoupled respiration and UCP2 and UCP3 gene expression. Hence, the phosphorylated amino acids must have altered PGC-1 α interfaces used for docking of specific transcription factors or other cofactors involved. We have shown previously [29] that inhibition of p38 MAPK abrogated the stimulation of UCP1 gene expression by PPAR α agonists in transiently transfected PAZ6 cells, but we now observed only minor effects of the p38 MAPK inhibitor SB 202190 on agonist-dependent and PGC-1 α -mediated co-activation of LXR in the same cell line (Table 1). Thus these results are consistent with unique phosphorylation codes that determine the functional specificity of PGC-1 α for distinct transcription factors [44].

The functioning of co-activators can be antagonized by selective repressor molecules that bind to them [45]. The binding surface of a putative repressor that competes with GR [28] and PPAR α [29] for binding to PGC-1 α has been mapped to the L3 motif. This motif is localized within a PGC-1 α region that has a repressive effect in transcription [46], serves as transcription-factor docking site and harbours the amino acids phosphorylated by p38 MAPK. Signalling via the p38 MAPK pathway reduces the interaction of PGC-1 α with the repressor and enhances co-activation of GR [28] and PPAR α [29]. The present study shows that PGC-1 α -mediated co-activation of LXR-dependent gene transcription is not affected by the repressor, even though the L2 motif of PGC-1 α is essential for co-activation of LXR, GR and PPAR α . This result is consistent with the modest decrease in LXR co-activation upon inhibition of p38 MAPK signalling, and it further supports the notion that activation of LXR and PPAR α is mediated by distinct PGC-1 α surfaces and/or depends on the recruitment of additional factors to the PGC-1 α complex.

Although the physiological relevance of LXR co-activation by PGC-1 α remains to be detailed, our findings suggest the potential role of PGC-1 α in plasma lipid transport. LXR agonists activate sterol-regulatory binding protein 1-c [16,17], ABCA1 [9], ABCG5 [14], ABCG8 [14], CETP [11] and lipoprotein lipase [47], all of which directly control specific aspects of lipoprotein metabolism. Previous studies in humans have shown the association of the PGC-1 α gene locus with obesity and Type II diabetes [48,49]. Whether the PGC-1 α or LXR α gene loci or an interaction between these loci affect plasma lipid transport in humans remains to be determined.

In conclusion, our results identify PGC-1 α as a potent transcriptional co-activator of LXRs in the presence of their cognate ligands. In addition, our studies identify p38 MAPK signalling and a putative inhibitor in the specificity of PGC-1 α for downstream effectors and raise the possibility that PGC-1 α plays a role in cellular cholesterol homeostasis and plasma lipid transport.

This study was supported by a biotechnology grant from the Bundesland Salzburg (Austria), by Jubilaeumsfondsprojekt no. 9364 from the Oesterreichische Nationalbank (Austria) and by a grant from the Medizinische Forschungsgesellschaft Salzburg (Austria).

REFERENCES

- Willy, P. J., Umesono, K., Ong, E. S., Evans, R. M., Heyman, R. A. and Mangelsdorf, D. J. (1995) LXR, a nuclear receptor that defines a distinct retinoid response pathway. *Genes Dev.* **9**, 1033–1045
- Song, C., Kokontis, J. M., Hiipakka, R. A. and Liao, S. (1994) Ubiquitous receptor: a receptor that modulates gene activation by retinoic acid and thyroid hormone receptors. *Proc. Natl. Acad. Sci. U.S.A.* **91**, 10809–10813
- Teboul, M., Enmark, E., Li, Q., Wikstrom, A. C., Pelto-Huikko, M. and Gustafsson, J. A. (1995) OR-1, a member of the nuclear receptor superfamily that interacts with the 9-*cis*-retinoic acid receptor. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 2096–2100
- Janowski, B. A., Willy, P. J., Devi, T. R., Falck, J. R. and Mangelsdorf, D. J. (1996) An oxysterol signaling pathway mediated by the nuclear receptor LXR α . *Nature (London)* **383**, 728–731
- Forman, B. M., Ruan, B., Chen, J., Schroeffer, Jr, G. J. and Evans, R. M. (1997) The orphan nuclear receptor LXR α is positively and negatively regulated by distinct products of mevalonate metabolism. *Proc. Natl. Acad. Sci. U.S.A.* **94**, 10588–10593
- Repa, J. J., Liang, G., Ou, J., Bashmakov, Y., Lobaccaro, J. M., Shimomura, I., Shan, B., Brown, M. S., Goldstein, J. L. and Mangelsdorf, D. J. (2000) Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXR α and LXR β . *Genes Dev.* **14**, 2819–2830
- Schultz, J. R., Tu, H., Luk, A., Repa, J. J., Medina, J. C., Li, L., Schwendner, S., Wang, S., Thoolen, M., Mangelsdorf, D. J., Lustig, K. D. and Shan, B. (2000) Role of LXRs in control of lipogenesis. *Genes Dev.* **14**, 2831–2838
- Peet, D. J., Turley, S. D., Ma, W., Janowski, B. A., Lobaccaro, J. M., Hammer, R. E. and Mangelsdorf, D. J. (1998) Cholesterol and bile acid metabolism are impaired in mice lacking the nuclear oxysterol receptor LXR α . *Cell (Cambridge, Mass.)* **93**, 693–704
- Costet, P., Luo, Y., Wang, N. and Tall, A. R. (2000) Sterol-dependent transactivation of the ABC1 promoter by the liver X receptor/retinoid X receptor. *J. Biol. Chem.* **275**, 28240–28245
- Laffitte, B. A., Repa, J. J., Joseph, S. B., Wilpitz, D. C., Kast, H. R., Mangelsdorf, D. J. and Tontonoz, P. (2001) LXRs control lipid-inducible expression of the apolipoprotein E gene in macrophages and adipocytes. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 507–512
- Luo, Y. and Tall, A. R. (2000) Sterol upregulation of human CETP expression *in vitro* and in transgenic mice by an LXR element. *J. Clin. Invest.* **105**, 513–520
- Kennedy, M. A., Venkateswaran, A., Tarr, P. T., Xenarios, I., Kudoh, J., Shimizu, N. and Edwards, P. A. (2001) Characterization of the human ABCG1 gene: liver X receptor activates an internal promoter that produces a novel transcript encoding an alternative form of the protein. *J. Biol. Chem.* **276**, 39438–39447
- Engel, T., Lorkowski, S., Lueken, A., Rust, S., Schluter, B., Berger, G., Cullen, P. and Assmann, G. (2001) The human ABCG4 gene is regulated by oxysterols and retinoids in monocyte-derived macrophages. *Biochem. Biophys. Res. Commun.* **288**, 483–488
- Repa, J. J., Berge, K. E., Pomajzl, C., Richardson, J. A., Hobbs, H. and Mangelsdorf, D. J. (2002) Regulation of ATP-binding cassette sterol transporters ABCG5 and ABCG8 by the liver X receptors α and β . *J. Biol. Chem.* **277**, 18793–18800
- Chawla, A., Repa, J. J., Evans, R. M. and Mangelsdorf, D. J. (2001) Nuclear receptors and lipid physiology: opening the X-files. *Science* **294**, 1866–1870
- DeBose-Boyd, R. A., Ou, J., Goldstein, J. L. and Brown, M. S. (2001) Expression of sterol regulatory element-binding protein 1c (SREBP-1c) mRNA in rat hepatoma cells requires endogenous LXR ligands. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 1477–1482
- Yoshikawa, T., Shimano, H., Amemiya-Kudo, M., Yahagi, N., Hasty, A. H., Matsuzaka, T., Okazaki, H., Tamura, Y., Iizuka, Y., Ohashi, K. et al. (2001) Identification of liver X receptor–retinoid X receptor as an activator of the sterol regulatory element-binding protein 1c gene promoter. *Mol. Cell. Biol.* **21**, 2991–3000
- Alberti, S., Schuster, G., Parini, P., Feltkamp, D., Diczfalussy, U., Rudling, M., Angelin, B., Bjorkhem, I., Pettersson, S. and Gustafsson, J. A. (2001) Hepatic cholesterol metabolism and resistance to dietary cholesterol in LXR β -deficient mice. *J. Clin. Invest.* **107**, 565–573
- Puigserver, P., Wu, Z., Park, C. W., Graves, R., Wright, M. and Spiegelman, B. M. (1998) A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell (Cambridge, Mass.)* **92**, 829–839
- Esterbauer, H., Oberkofler, H., Krempler, F. and Patsch, W. (1999) Human peroxisome proliferator activated receptor γ coactivator 1 (PPARGC1) gene: cDNA sequence, genomic organization, chromosomal localization, and tissue expression. *Genomics* **62**, 98–102
- Lin, J., Wu, H., Tarr, P. T., Zhang, C. Y., Wu, Z., Boss, O., Michael, L. F., Puigserver, P., Isotani, E., Olson, E. N. et al. (2002) Transcriptional co-activator PGC-1 α drives the formation of slow-twitch muscle fibres. *Nature (London)* **418**, 797–801
- Lin, J., Puigserver, P., Donovan, J., Tarr, P. and Spiegelman, B. M. (2002) Peroxisome proliferator-activated receptor γ coactivator 1 β (PGC-1 β), a novel PGC-1-related transcription coactivator associated with host cell factor. *J. Biol. Chem.* **277**, 1645–1648
- Kressler, D., Schreiber, S. N., Knutti, D. and Kralli, A. (2002) The PGC-1-related protein PERC is a selective coactivator of estrogen receptor α . *J. Biol. Chem.* **277**, 13918–13925
- Wu, Z., Puigserver, P., Andersson, U., Zhang, C., Adelman, G., Mootha, V., Troy, A., Cinti, S., Lowell, B., Scarpulla, R. C. et al. (1999) Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell (Cambridge, Mass.)* **98**, 115–124

- 25 Michael, L. F., Wu, Z., Cheatham, R. B., Puigserver, P., Adelmant, G., Lehman, J. J., Kelly, D. P. and Spiegelman, B. M. (2001) Restoration of insulin-sensitive glucose transporter (GLUT4) gene expression in muscle cells by the transcriptional coactivator PGC-1. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 3820–3825
- 26 Herzig, S., Long, F., Jhala, U. S., Hedrick, S., Quinn, R., Bauer, A., Rudolph, D., Schutz, G., Yoon, C., Puigserver, P. et al. (2001) CREB regulates hepatic gluconeogenesis through the coactivator PGC-1. *Nature (London)* **413**, 179–183
- 27 Yoon, J. C., Puigserver, P., Chen, G., Donovan, J., Wu, Z., Rhee, J., Adelmant, G., Stafford, J., Kahn, C. R., Granner, D. K. et al. (2001) Control of hepatic gluconeogenesis through the transcriptional coactivator PGC-1. *Nature (London)* **413**, 131–138
- 28 Knutti, D., Kressler, D. and Kralli, A. (2001) Regulation of the transcriptional coactivator PGC-1 via MAPK-sensitive interaction with a repressor. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 9713–9718
- 29 Oberkofler, H., Esterbauer, H., Linnemayr, V., Strosberg, A. D., Krempler, F. and Patsch, W. (2002) Peroxisome proliferator-activated receptor (PPAR) γ coactivator-1 recruitment regulates PPAR subtype specificity. *J. Biol. Chem.* **277**, 16750–16757
- 30 Laffitte, B. A., Joseph, S. B., Walczak, R., Pei, L., Wilpitz, D. C., Collins, J. L. and Tontonoz, P. (2001) Autoregulation of the human liver X receptor α promoter. *Mol. Cell. Biol.* **21**, 7558–7568
- 31 Whitney, K. D., Watson, M. A., Goodwin, B., Galardi, C. M., Maglich, J. M., Wilson, J. G., Willson, T. M., Collins, J. L. and Kliewer, S. A. (2001) Liver X receptor (LXR) regulation of the LXR α gene in human macrophages. *J. Biol. Chem.* **276**, 43509–43515
- 32 Zilberfarb, V., Pietri-Rouxel, F., Jockers, R., Krief, S., Delouis, C., Issad, T. and Strosberg, A. D. (1997) Human immortalized brown adipocytes express functional β 3-adrenoceptor coupled to lipolysis. *J. Cell Sci.* **110**, 801–807
- 33 Esterbauer, H., Schneitler, C., Oberkofler, H., Ebenbichler, C., Paulweber, B., Sandhofer, F., Ladurner, G., Hell, E., Strosberg, A. D., Patsch, J. R. et al. (2001) A common polymorphism in the promoter of UCP2 is associated with decreased risk of obesity in middle-aged humans. *Nat. Genet.* **28**, 178–183
- 34 Vega, R. B., Huss, J. M. and Kelly, D. P. (2000) The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor α in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. *Mol. Cell. Biol.* **20**, 1868–1876
- 35 Knutti, D., Kaul, A. and Kralli, A. (2000) A tissue-specific coactivator of steroid receptors, identified in a functional genetic screen. *Mol. Cell. Biol.* **20**, 2411–2422
- 36 Tcherepanova, I., Puigserver, P., Norris, J. D., Spiegelman, B. M. and McDonnell, D. P. (2000) Modulation of estrogen receptor- α transcriptional activity by the coactivator PGC-1. *J. Biol. Chem.* **275**, 16302–16308
- 37 Wu, Y., Delerive, P., Chin, W. W. and Burris, T. P. (2002) Requirement of helix 1 and the AF-2 domain of the thyroid hormone receptor for coactivation by PGC-1. *J. Biol. Chem.* **277**, 8898–8905
- 38 Heery, D. M., Kalkhoven, E., Hoare, S. and Parker, M. G. (1997) A signature motif in transcriptional co-activators mediates binding to nuclear receptors. *Nature (London)* **387**, 733–736
- 39 Orphanides, G. and Reinberg, D. (2002) A unified theory of gene expression. *Cell (Cambridge, Mass.)* **108**, 439–451
- 40 Delerive, P., Wu, Y., Burris, T. P., Chin, W. W. and Suen, C. S. (2002) PGC-1 functions as a transcriptional coactivator for the retinoid X receptors. *J. Biol. Chem.* **277**, 3913–3917
- 41 Huss, J. M., Kopp, R. P. and Kelly, D. P. (2002) PGC-1 α coactivates the cardiac-enriched nuclear receptors ERR α and γ via novel leucine-rich interaction interfaces. *J. Biol. Chem.* **277**, 40265–40274
- 42 Knutti, D. and Kralli, A. (2001) PGC-1, a versatile coactivator. *Trends Endocrinol. Metab.* **12**, 360–365
- 43 Puigserver, P., Rhee, J., Lin, J., Wu, Z., Yoon, J. C., Zhang, C. Y., Krauss, S., Mootha, V. K., Lowell, B. B. and Spiegelman, B. M. (2001) Cytokine stimulation of energy expenditure through p38 MAP kinase activation of PPAR γ coactivator-1. *Mol. Cell* **8**, 971–982
- 44 McKenna, N. J. and O'Malley, B. W. (2002) Combinatorial control of gene expression by nuclear receptors and coregulators. *Cell (Cambridge, Mass.)* **108**, 465–474
- 45 Lee, C. H., Chinpaisal, C. and Wei, L. N. (1998) Cloning and characterization of mouse RIP140, a corepressor for nuclear orphan receptor TR2. *Mol. Cell. Biol.* **18**, 6745–6755
- 46 Puigserver, P., Adelmant, G., Wu, Z., Fan, M., Xu, J., O'Malley, B. and Spiegelman, B. M. (1999) Activation of PPAR γ coactivator-1 through transcription factor docking. *Science* **286**, 1368–1371
- 47 Zhang, Y., Repa, J. J., Gauthier, K. and Mangelsdorf, D. J. (2001) Regulation of lipoprotein lipase by the oxysterol receptors, LXR α and LXR β . *J. Biol. Chem.* **276**, 43018–43024
- 48 Esterbauer, H., Oberkofler, H., Linnemayr, V., Iglseder, B., Hedegger, M., Wolfgruber, P., Paulweber, B., Fastner, G., Krempler, F. and Patsch, W. (2002) Peroxisome proliferator-activated receptor- γ coactivator-1 gene locus: associations with obesity indices in middle-aged women. *Diabetes* **51**, 1281–1286
- 49 Ek, J., Andersen, G., Urhammer, S. A., Gaede, P. H., Drivsholm, T., Borch-Johnsen, K., Hansen, T. and Pedersen, O. (2001) Mutation analysis of peroxisome proliferator-activated receptor- γ coactivator-1 (PGC-1) and relationships of identified amino acid polymorphisms to Type II diabetes mellitus. *Diabetologia* **44**, 2220–2226

Received 24 October 2002/6 December 2002; accepted 9 December 2002

Published as BJ Immediate Publication 9 December 2002, DOI 10.1042/BJ20021665