Characterization of the human, mouse and rat PGC1 β (peroxisome-proliferator-activated receptor- γ co-activator 1 β) gene *in vitro* and *in vivo*

Aline MEIRHAEGHE^{*1}, Vivion CROWLEY^{*1}, Carol LENAGHAN^{†1}, Christopher LELLIOTT^{*}, Kath GREEN[‡], Abigail STEWART^{*}, Kevin HART[†], Sven SCHINNER^{*}, Jaswinder K. SETHI^{*}, Giles YEO^{*}, Martin D. BRAND[‡], Ron N. CORTRIGHT[§], Stephen O'RAHILLY^{*}, Carl MONTAGUE[†] and Antonio J. VIDAL-PUIG^{*2}

^{*}Departments of Clinical Biochemistry and Medicine, Cambridge Institute for Medical Research, University of Cambridge, Addenbrooke's Hospital, Hills Road, Cambridge, U.K., [†]Cardiovascular and Gastrointestinal Discovery Research Department, AstraZeneca Pharmaceuticals, Macclesfield, U.K., [‡]MRC Dunn Human Nutrition Unit, Cambridge, U.K., and [§]Departments of Exercise, Sport Science and Physiology, East Carolina University, Greenville, NC 27858, U.S.A.

PGC1 α is a co-activator involved in adaptive thermogenesis, fatty-acid oxidation and gluconeogenesis. We describe the identification of several isoforms of a new human PGC1 α homologue, cloned independently and named PGC1 β . The human PGC1 β gene is localized to chromosome 5, has 13 exons and spans more than 78 kb. Two different 5' and 3' ends due to differential splicing were identified by rapid amplification of cDNA ends PCR and screening of human cDNA libraries. We show that PGC1 β variants in humans, mice and rats are expressed predominantly in heart, brown adipose tissue, brain and skeletal muscle. PGC1 β expression, unlike PGC1 α , is not up-regulated in brown adipose tissue in response to cold or obesity. Fasting experiments showed that PGC1 α , but not PGC1 β , is induced in liver and this suggests that only PGC1 α is

involved in the hepatic gluconeogenesis. No changes in PGC1 β gene expression were observed associated with exercise. Human PGC1 β -1a and -2a isoforms localized to the cell nucleus and, specifically, the isoform PGC1 β -1a co-activated peroxisome-proliferator-activated receptor- γ , - α and the thyroid hormone receptor β 1. Finally, we show that ectopic expression PGC1 β leads to increased mitochondrial number and basal oxygen consumption. These results suggest that PGC1 β may play a role in constitutive adrenergic-independent mitochondrial biogenesis.

Key words: gene expression, genomic structure, isoform, mitochondrial biogenesis, peroxisome-proliferator-activated receptor- γ co-activator 1 α (PGC1 α), PGC1 β .

INTRODUCTION

Nuclear receptor (NR) co-activators modulate transcriptional activity either by introducing enzymic modifications to the chromatin of promoter DNA or by facilitating the recruitment of the transcription machinery to specific promoters [1,2]. In addition, co-activators provide specificity by targeting tissue- and cell-type-specific subsets of NRs to specific promoters. Peroxisome-proliferator-activated receptor- γ co-activator 1 α (PGC1 α) is one such co-activator of the NR peroxisome-proliferator-activated receptor- γ (PPAR γ), a transcription factor that plays key roles in processes such as adipogenesis [3] and energy expenditure [4]. PGC1 α was described originally as a brown-fat-specific co-activator isolated through a yeast two-hybrid strategy using a PPAR γ as bait and a brown fat cDNA library. Initial reports showed that PGC1 α was expressed highly in brown adipose tissue (BAT) [4], and that PGC1 α was the key factor that directed PPAR γ towards the subset of genes that regulated a programme of adaptive thermogenesis. It was also speculated that another primary role of PGC1 α was to co-activate PPAR γ to induce the expression of genes leading to the differentiation of preadipocytes into brown adipocytes [4]. More recently, it has been shown that PGC1 α demonstrates highly selective tissue expression and

that it is capable of interacting with and co-activating other NRs, e.g. thyroid receptor (TR), hepatocyte nuclear factor (HNF)-4 α [5,6], endoplasmic reticulum [7], PPAR α [8], retinoid X receptors (RXRs) [9] and non-NR transcription factors such as nuclear respiratory factor 1 (NRF-1) [10] and myocyte-specific enhancer-binding factor 2 transcription factor [11], indicating that PGC1 α is involved in a more broad spectrum of actions than suspected initially [12].

Regulation of the activity of NR co-activators may occur through different mechanisms including translocation between cytoplasm and nucleus, post-translational modifications, e.g. phosphorylation and acetylation, and regulated proteolysis [13]. PGC1 α is also highly regulated at the transcriptional level in response to specific physiological stimuli which is unusual for a co-activator. For instance, cold exposure induces PGC1 α gene expression in BAT and skeletal muscle. This is associated with stimulation of mitochondrial uncoupled respiration through the induction of uncoupling proteins [14,15] and activation of the mitochondrial biogenesis programme through its co-activating activity on NRF-1 [4,10]. PGC1 α gene expression is also induced markedly during fasting in mouse hepatocytes through a mechanism that involves cAMP-response-element-binding protein phosphorylation [6]. PGC1 α plays an important role in activating gluconeogenesis by co-activating HNF-4 α

Abbreviations used: BAT, brown adipose tissue; CHO, Chinese-hamster ovary; DMEM, Dulbecco's modified Eagle's medium; FBS, foetal bovine serum; FCCP, carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone; GFP, green fluorescent protein; GRE, glucocorticoid response element; HNF, hepatocyte nuclear factor; IRES, internal ribosomal entry; NR, nuclear receptor; NRF-1, nuclear respiratory factor 1; PERC, PGC1 (peroxisome-proliferator-activated receptor- γ co-activator 1)-related estrogen receptor co-activator; PPAR, peroxisome-proliferator-activated receptor; PRC, PGC-1-related co-activator; RACE, rapid amplification of cDNA ends; RPA, RNase protection assay; TEM, transmission electron microscopy; T₃, 3,3',5-tri-iodothyronine; TR, thyroid receptor; RXR, retinoid X receptor.

¹ These authors have contributed equally to this work.

² To whom correspondence should be addressed (e-mail ajv22@cam.ac.uk).

and glucocorticoid receptor on the phosphoenolpyruvate carboxykinase promoter [5,6]. Fasting also induces the expression of PGC1 α in heart suggesting that it may contribute to fatty-acid oxidation through its co-activating action on PPAR α [16,17]. Thus PGC1 α is now considered a versatile co-activator [12] that plays important roles not only in adaptative thermogenesis in brown fat and skeletal muscle [4], but also in the gluconeogenic response associated with fasting [5,6].

Recently, two new co-activators have been added to this family. One of them is PGC1 β [18] and the human orthologue of this is PGC-1-related estrogen receptor co-activator (PERC), which has been described as a selective co-activator of the oestrogen receptor α [19]. The second is PGC-1-related co-activator (PRC) [20], an ubiquitously expressed PGC1-related co-activator, which may play a role in modulating mitochondrial biogenesis during proliferation. This family of co-activators shares [21] maximum similarity at the N- and C-terminal regions, areas that carry most of the effector domains. Common elements in their structure are the NR boxes containing leucine-rich motifs (LXXLL domains), which mediate the interaction with hormone receptor-binding domains, an RNA-binding motif and, in PGC1 α and PRC, a serine/arginine-rich domain that regulates RNA processing [22]. Recently, the presence of host cell factor motifs has also been described, which may link these co-activators with processes involving cellular proliferation/differentiation [18].

PGC1 α provides the molecular basis for an integrated model of transcriptional control of metabolic-specific programmes. We speculated, independently, on the possible existence of other PGC1 α homologues that may collaborate to integrate the same or other gene-specific transcription programmes. The present study was concerned with the novel human PGC1 α homologue, which has also been reported as PERC and the mouse orthologue PGC1 β . We report the human PGC1 β cDNA sequence including several N- and C-terminal splice isoforms, which appear to be conserved in rodents. We describe the genomic structure of human PGC1 β and the basis for its alternatively spliced variants. The PGC1 β mRNA tissue distribution in humans, rats and mice is highly conserved and PGC1 β , in contrast with PGC1 α , does not seem to be regulated transcriptionally *in vivo* under the experimental conditions tested. Our results suggest that it is only PGC1 α that is up-regulated in liver during fasting. *In vitro* results provide evidence that PGC1 β is localized in the nucleus, where it co-activates several transcription factors. Moreover, when PGC1 β is overexpressed ectopically in L6 myoblasts, there is an increased number of mitochondria and oxygen consumption suggesting that PGC1 β may play a role in constitutive non-adrenergic-mediated mitochondrial biogenesis.

EXPERIMENTAL

Rapid amplification of cDNA ends (RACE) PCRs

The primer 5'-GTCACAAAGCGACCCAACTT-3' (nt 1671–1691) was used for the 3'-RACE PCR using the human adipocyte Marathon Ready cDNA and the AP1 adaptor primer (ClonTech, Basingstoke, Hants. U.K.) in accordance with the manufacturer's instructions. Full-length cDNA and 5' variation in human PGC1 β were determined using the 5'/3'-RACE Kit (Roche Diagnostics, Basel, Switzerland). Total RNA from adult female human heart (Stratagene, Cedar Creek, TX, U.S.A.) was used for first-strand synthesis of cDNA with the primer 5'-CATCA-CAGAGCACGTCTTGAG-3'. Subsequently, an oligo(dT) primer (supplied by the manufacturer) and PGC1 β -specific antisense primer 5'-CATGTAGCGTATGAGTTGCACCATC-3' were used to amplify the sequence between exon 5 and the putative 5'

terminal, and this was followed by a further round of PCR using the oligo(dT) primer and a nested PGC1 β exon 5'-specific primer 5'-GCCGAGAGGTGCTTATGTAGTTC-3' (minimum expected size 605 bp). All PCR products were visualized on 1.2 % (w/v) agarose gel and all fragments > 500 bp were purified subsequently and then cloned into pBlueScript (Stratagene) using standard techniques. Multiple clones were screened and sequenced. A second 5'-RACE using adult human subcutaneous abdominal mature adipocyte RNA for first-strand synthesis was also performed as described above (see the Results section).

Isolation of the full-length clone

We screened a human heart cDNA library master plate (Origene LHT-1001; OriGene Technologies, Rockville, MD, U.S.A.) with the PCR generated by the following oligonucleotides: 5'-GCCACTCGAAGGAAGTTCAGAT-3' (nt 2536–2558) and 5'-GGGTAAAGGCTGTTATCAATGC-3' (nt 3003–3025 of the PGC1 β -a isoform). Several wells were found to be positive and the subplates corresponding to those wells were obtained from OriGene Technologies. Clones containing PGC1 β -a were identified and sequenced. This resulted in the isolation of the complete cDNA sequence for PGC1 β -2a.

RNA extraction

Mice and rat RNAs were extracted from tissues using the STAT-60 method (Tel-Test 'B', Friendwood, TX, U.S.A.) or TRIzol[®] reagent (Gibco BRL, Invitrogen, Paisley, U.K.) according to the manufacturer's instructions. RNA concentration and integrity were determined using a spectrophotometer at 260 nm and an ethidium bromide-stained agarose gel respectively. Samples were stored at –80 °C until processed. Human RNAs were obtained from BioChain (Hayward, CA, U.S.A.).

RNase protection assay (RPA)

Probe synthesis

RPAs were performed as described previously [23–25]. The isoform-specific probes were amplified by PCR (see oligonucleotides and conditions in Table 1) and cloned into the vector pGEM-T easy (Promega) in the orientation 3'–5'. The identity of the sequence was confirmed by sequencing. The plasmids were linearized with *Spe*I. The ribonucleotide probe was synthesized with the T7 polymerase (Stratagene) using 300 ng of vector, 4 μ l of [α -³²P]UTP (Amersham Biosciences), rATP, rGTP and rCTP. Probes were purified from mononucleotides by ethanol precipitation in the presence of 2.5 M ammonium acetate. Probe integrity was determined by autoradiography after electrophoresis on 4 % (w/v) polyacrylamide gel/7 % (w/v) urea-denaturing gel. The assays were done with 10 μ g of RNA using 0.2–0.3 ng of PGC1 α and PGC1 β or 0.2–2 ng of cyclophilin-labelled probe (Ambion, Austin, TX, U.S.A.).

Solution hybridization RPA

RNA transcripts were quantified using solution hybridization RPA methods as described previously [25]. In brief, [³²P]UTP-labelled RNA probes were incubated with total RNA samples in a final volume of 25 μ l of hybridization solution for 12–16 h at 30 °C. Samples defined as free probe include only RNA-labelled probe treated with RNases. Samples were then incubated for 1 h at 30 °C with a mixture of RNase A (40 μ g/ml) and RNase

Table 1 RNase Protection Probes

Abbreviation: WAT, white adipose tissue.

Species/name	Location in the gene	Size (bp)	Primer F (5' → 3')	Primer R (5' → 3')	Annealing temperature (°C)	Tissue of origin
Human						
Middle	Part of exon 5	300	CAAGACAAGAAGGCTCCCATGA	GCAGTAGGTGTCATGTAGCGTA	53	WAT
C-terminus	Part of exon 10, exon 11a and part of exon 12 (52 bp of PGC-1 β a)	252	AGTGTTTGGTGAGATTGAGGAGTGCGAGGT	TCATACTTGCTTTTCCCTGACGCAGGAAG	52	WAT
N-terminus	Part of exon 1a (110 bp = PGC-1 β 1 isoform) + 150 bp exon 2	260	ACTCCGCCGCACGCTGCAGC	GGCTGTACTGGTTGGCTTC	52	5' RACE PCR on heart
Mouse						
C-terminus	Part of exon 10, exon 11a and part of exon 12 (49 bp of PGC-1 β a)	252	GGTGTCGGTGAGATTGAGAGTGCCAGGT	TCGTA CTGCTTTTCCAGATGAGGGAAG	52	Liver
Middle	Part of exon 8, exons 9, 10 and 11a	398	ATAGTCAGTGATCTGG	CTCGTCCTGCAGCTCCTG	51	Liver
Rat						
N-terminus	56 bp 5'UTR + 204 bp exon 2 and part of exon 3	260	GCGGAAGGACTATCTCGG	CTCCATGTCAATCTGGAAG	51	Liver
C-terminus	Part of exon 10, exon 11a and part of exon 12 (59 bp of PGC-1 β a)	263	AGGTGTTTGGTGAGATAGTCG	ATCCATGGCTTCGTACTTGC	53	Liver
Mouse PGC1 α	Nucleotide 2091–2339	249	AGTTTTTGGTGAAATTGAGGAAT	TCATACTTGCTCTTGGTGGAAGC	55	Expression vector

T1 (2 μ g/ml; Sigma). To inactivate the nucleases, samples were treated with 10% (w/v) SDS (20 μ l) and 10 mg/ml proteinase K (5 μ l) for 20 min at 45 °C and extracted with phenol/chloroform. After precipitation with ethanol and dissolution in 7 μ l of gel-loading buffer [25% (w/v) Ficoll, 0.2 M EDTA (pH 8), 0.25% Bromophenol Blue and 0.25% Xylene Cyanole], samples were resolved on 5% (w/v) non-denaturing polyacrylamide gels (0.75 mm thick) at 300 V for 2 h. Gels were mounted on 3 M (Whatman Inc., Clifton, NJ, U.S.A.) paper and dried. Protected bands were visualized by autoradiography and quantified by PhosphorImager analysis using ImageQuant software (Molecular Dynamics, Sunnyvale, CA, U.S.A.).

In vivo experiments

Animals were housed in a temperature-controlled room with a 12 h:12 h light/dark cycle. Food and water were available *ad libitum* unless noted. All experiments were conducted in accordance with the Home Office Guidelines for the Care and Use of Laboratory Animals. Mice (C57BL/6) and rats (Wistar and Zucker rats) were subjected to specific nutritional perturbation protocols as indicated in the Results section. High-fat diet experiments were performed in male rats, which had received RM1 chow pellets from Special Diet Services (Witham, Essex, U.K.) in addition to the stated diets. The low-fat diet was powdered RM1 diet from Special Diet Services (2–4% fat) and the high-fat diet was Harlan Teklad TD 88137 (42% fat). All animals remained on their diet for 10 weeks. Then, they were either fasted for 17–21 h or fed before being killed. Liver and heart tissues were taken. Fasting/refeeding experiments were performed in Wistar rats and C57BL/6 mice. RNA from cold-exposure experiments was provided by Dr C. Y. Zhang (Harvard University Boston, MA, U.S.A.). The effect of exercise was tested using an acute endurance exercise protocol performed at East Carolina University. C57BL/6 wild-type mice were obtained from Harlan Laboratories (St. Louis, MO, U.S.A.). Twenty-six mice were randomly assigned to either a sedentary or exercised group and the latter was accustomed to treadmill running over 2 days. On the exercise day, the exercised

animals were run for 3 h at a treadmill speed of 16–18 m/min at 0% grade, rested for 1 h and then run again for 3 h. Animals were killed 12 h later by CO₂ asphyxiation and whole gastrocnemius was removed (within seconds), immediately frozen with liquid nitrogen-cooled stainless-steel tongs, and stored at –80 °C for subsequent mRNA extraction.

In vitro experiments

Transient transfections

HepG2 cells were maintained with Dulbecco's modified Eagle's medium (DMEM), 4.5 g of glucose/l (Sigma), 10% foetal bovine serum (FBS) and penicillin/streptomycin. Cells were split into 24-well plates and transfected 8 h later in the same medium using FuGENE (Roche, Basel, Switzerland). A ratio of 3 μ l FuGENE/1 μ g DNA was used for all the transfections. The transfection efficiency was estimated by co-transfecting the vector pRL-CMV (Promega) that encodes the *Renilla* luciferase gene. Fifteen hours after transfection, the medium was removed, the cells were washed once with PBS and fresh medium containing the adequate ligands was added for 30 h. Then, the cells were harvested and both firefly and *Renilla* luciferase activities were measured into 20 μ l of lysate according to the manufacturer's instructions (Dual Luciferase assay; Promega). The compound GW327647 was obtained from GlaxoSmithKline (Glaxo Wellcome UK Ltd., Stockley Park West, Uxbridge, Middlesex, U.K.) and used at 100 nM as a specific PPAR α ligand. The compound LG100268 (at 100 nM) was used as an RXR α agonist and BRL49653 (at 100 nM) was used as a PPAR γ agonist. In the case of TR β 1, 3,3',5-tri-iodothyronine (T₃) was used at 100 nM and 1 μ M. Chinese-hamster ovary (CHO) cells were maintained in Ham-F12 medium (Sigma) plus 10% (v/v) FBS and penicillin–streptomycin. CHO cells in a six-well plate were transfected with 1.5 μ g of pGEN GFP-PGC-1 β or pGEN GFP (where GFP stands for green fluorescent protein) alone. The fluorescence was assessed 48 h later under a microscope.

The HeLa cell line was transfected stably with a glucocorticoid response element (GRE)- β -galactosidase reporter construct. The resultant cell line, termed GRE 4- β -gal HeLa, was maintained in

DMEM with 10% charcoal-stripped FBS and gentamicin. The GRE- β -gal reporter cell line was transfected transiently using a calcium phosphate transfection kit (Invitrogen, Carlsbad, CA, U.S.A.) according to the standard method. A total of 20 μ g of DNA was transfected per T75 flask of cells at 50% confluency. Control transfections with a luciferase-control plasmid, pGL3 (Promega), to monitor transfection efficiency and pcDNA3 carrier DNA were compared with transfections containing pcDNA3-PGC1 β -1a, pGL3 and carrier pcDNA3. After 24 h, the transfected transiently cells were seeded at 1×10^5 cells/well into a 96-well plate. After 2 h, cells were stimulated with a submaximal concentration (1 nM) of dexamethasone or left unstimulated. At 48 h post-transfection, the cells were assayed for β -galactosidase and firefly luciferase using a Dual-Light kit (Tropix, Bedford, MA, U.S.A.).

Plasmid constructs

Full-length human PGC1 β -1a and -2a cDNA clones were obtained by ligating the respective 5' ends of PGC1 β cDNAs generated by 5'-RACE (5'-PGC1 β -1a and -2a) to the PGC1 β -a 3' isoform isolated from human heart cDNA library (as outlined above). In essence, amplicons of 412 and 578 bp incorporating an *Eco*RI restriction digest site were generated by PCR using *Pfu* turbo as polymerase and pBS-5'-PGC1 β -1a and -PGC1 β -1 as respective templates. These amplicons extended from exon 3 to the 5'-terminus on exon 1a, common to both PGC1 β -1a and -2a. Both amplicons underwent subsequent sequential restriction digest with *Eco*RI and *Psh*AI, followed by ligation into *Eco*RI-*Psh*AI sites of pGEN-IRES-PGC1 β -1a (where IRES stands for internal ribosomal entry). A further digest of both pGEN-IRES-PGC1 β -1a and PGC1 β -2a using an *Eco*RI-*Xba*I combination was followed by ligation into *Eco*RI-*Xba*I sites of pcDNA3, thus generating isoform-specific vectors for use in transient transfection assay systems.

To generate the retroviral expression vectors, *Eco*RI-*Xba*I inserts containing full-length PGC1 β -1a and -2a were released from both pGEN-IRES-PGC1 β -1a and -2a respectively, and treated with T4 DNA polymerase to generate blunt-ended fragments. A subsequent blunt-ended ligation of the respective PGC1 β -1a and -2a *Eco*RI-*Xba*I fragments into the *Sna*BI site of pBabe-puromycin (a gift from Dr B. M. Spiegelman, Harvard University) was performed using standard methods. pBabe-mPGC1 α was generated by initial ligation of a *Sma*I-*Not*I fragment from pSV-Sport-mPGC1 α (a gift from Dr B. M. Spiegelman) with subsequent blunt-ended ligation of this fragment into the *Sna*BI site of pBabe-puromycin, as described above. All plasmid constructs were verified by direct sequencing.

Myoblast cell culture and retroviral infection

Rat L6 myoblast cell lines (a gift from Dr Justin Rochford, University of Cambridge, Cambridge, U.K.) were maintained in DMEM containing 10% FBS (Sigma). To generate cell lines stably expressing the construct of interest, these myoblasts were infected with retrovirus containing either pBabe-puromycin, pBabe-mPGC1 α , pBabe-hPGC1 β -1a or pBabe-PGC1 β -2a as appropriate, prepared as per the methods described previously [4]. Following selection with puromycin (4 μ g/ml), the residual virally infected cells were maintained in culture. To induce myotube differentiation, these stable myoblasts were grown to confluence and cultured subsequently in minimum essential medium α + Glutamax (Gibco BRL) medium with 2% (v/v) horse serum added for a period of 5 days.

Transmission electron microscopy (TEM)

Cells were prepared for ultra-structural analysis using TEM as described previously [26]. Briefly, the cells were fixed in 2.5% (w/v) glutaraldehyde and 2% (w/v) paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 1 h at room temperature (24 °C). After washing the cells in 0.1 M sodium cacodylate buffer, the cells were fixed in 1% (w/v) osmium tetroxide in 0.1 M sodium cacodylate buffer for 1 h at room temperature. The cells were then washed as before, dehydrated through a graded ethanol series and infiltrated with Agar 100 resin, before embedding and curing in Agar 100 at 60 °C for 48 h. Sections were cut on a Leica Ultracut microtome, contrasted with 3% (w/v) aqueous uranyl acetate and lead citrate, and viewed using a Philips CM 100 TEM. We used point counting to calculate the percentage of cell volume containing mitochondria [27]. Using AnalySis image analysis software (SIS, Scientific Imaging Software; Scanalytics, Fairfax, VA, U.S.A.) in conjunction with a MegaView digital camera on the TEM, a fixed 1000 μ m $\times$ 1000 μ m grid was placed over the images and mitochondria that lay on any intersecting points of the grid were scored. This gave the ratio of mitochondria per unit cytoplasm and, hence, the percentage of the cell containing mitochondria.

Measurement of oxygen consumption in rat L6 myoblasts

For oxygen consumption experiments, cells were detached from plates using trypsin and then centrifuged and resuspended in DMEM containing 25 mM Hepes. The suspension was transferred immediately to the 1 ml chamber of a Clarke-type oxygen electrode maintained at 37 °C. Oxygen consumption readings were recorded using Powerlab (AD Instruments, Charlotte, NC, U.S.A.). The uninhibited rate was determined by measuring the linear rate of oxygen consumption. Oligomycin (5 μ g/ml) and 4 μ M carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) were added sequentially to the chamber, and the oxygen consumption was recorded after each addition. Myxothiazol (5 μ M)-inhibited rates were extremely low (<1% basal) and similar for each cell type, excluding the possibility that non-mitochondrial oxygen consumption rates differed between cell lines. The myxothiazol-inhibited rate was subtracted from the basal, oligomycin and FCCP rates. Once measurements were complete, cells were collected from the chamber and counted using a haemocytometer (Neubauer Improved; Weber Scientific, Hamilton, NJ, U.S.A.), taking the average of two counts of over 50 cells.

RESULTS

Characterization of human, mouse and rat PGC1 β cDNA and protein

From the Incyte LifeSeq Gold database, we identified a cDNA sequence derived from a human adipose tissue library with 30% homology to the C-terminus of human and mouse PGC1 α (Incyte clone ID no. 1956431). This clone was shown subsequently to be similar to PERC [19] and to be orthologous to the mouse gene PGC1 β [18]. As this Incyte clone was incomplete, and also to identify potential new 3'-splice variants of PGC1 β , a 3'-RACE PCR analysis was conducted. A 1.5 kb PCR product was amplified from human adipose tissue cDNA and sequenced. This 3'-RACE product was found to be similar but not identical at the most 3'-end when compared with the original Incyte clone (Figure 1A). Consequently, we determined that at least two C-terminal variants of human PGC1 β exist and these have been termed PGC1 β -a and -b based on sequence homology with

Figure 1 Characterization of the human PGC1 β cDNA and protein

(A) Schematic diagram outlining the 3'-isoform cDNA sequences of PGC1 β identified using 3'-RACE PCR; the coding region is represented by solid boxes, the non-coding region of exons 11b and 12 are both represented by dotted boxes. Alternative splice site of exon 11 is illustrated by the broken line. (B) Alignment of amino acid sequences of the C-terminus of PGC1 β -a and -b. (C) Schematic diagram illustrating the 5'-isoform cDNA sequences of PGC1 β -1 and -2 isolated using 5'-RACE PCR; coding region is represented by solid boxes, the non-coding region of exon 1a by the dotted box and the non-coding region of exon 1b by the hatched box. Note that the translational start site in exon 1a is not in-frame in the longer PGC1 β -2 isoform. (D) Alignment of the amino acid sequences of the N-termini of PGC1 β -1 and -2. (E) Putative genomic organization of PGC1 β gene. Exons are represented as open boxes and introns as solid black lines. The size of the introns are indicated in italics above the introns. The size (bp) of the exons are indicated in the boxes. The LXXLL motif is positioned in exon 4.

PGC-1 α (see below). Figure 1(B) illustrates that at the extreme C-terminus, PGC1 β -a encodes 33 amino acids not found in PGC1 β -b, whereas the latter isoform encodes 27 amino acids, which differ with respect to PGC1 β -a. Moreover, sequence alignment reveals no similarity between the two isoforms at this site.

Since the expression of PGC1 β was more abundant in heart (see below), we screened a human heart cDNA library for PGC1 β clones. A clone containing the putative full-length cDNA of PGC1 β -a was isolated and sequenced. We also proceeded to characterize the N-terminus of PGC1 β using 5'-RACE PCR on human heart and adipose-tissue total RNA. Direct sequencing of cDNAs identified by 5'-RACE (see the Experimental section) revealed two distinct isoforms of PGC1 β with variation at the N-terminus. More specifically, all clones isolated during the analysis using total RNA from human heart represented a single isoform (PGC1 β -1), whereas the analysis using RNA from human subcutaneous mature adipocytes identified a second, longer cDNA (PGC1 β -2) containing an extra 166 nt sequence, in addition to identifying the PGC1 β -1 isoform (Figures 1C and 1D).

In conjunction with the 5'- and 3'-RACE, we utilized the NCBI BLAST program to search for PGC1 β cDNA sequence homologues in the human genome database and located an exact match on a chromosome 5 BAC clone (AC008545, clone CTC-500L4). Thus a putative full-genomic structure of human PGC1 β gene was determined and this indicated that the gene consists of 13 exons and spans more than 78 kb (Figure 1E). Only exon 1a could not be found on the chromosome 5 BAC clone, suggesting that intron 1a is greater than 48 kb. Moreover, this genomic organization suggests strongly that exon 1b can be spliced out, generating the PGC1 β -1 isoform, whereas PGC1 β -a and -b are generated by alternative splicing of exon 11. Interestingly, the putative translational start site for PGC1 β -1 is also present in the PGC1 β -2 sequence, although in the latter it does not provide an open reading frame. However, PGC1 β -2 does feature a second downstream in-frame translational start site (Figure 1C). The PGC1 β -1 transcript generates a protein of 1023 and 1017 amino acids when paired with the a and b 3'-isoforms respectively, whereas the longer PGC1 β -2 transcript actually encodes a protein 21 amino acids shorter than PGC1 β -1 when paired with the same respective C-termini (3'a, 1002 and 3'b, 996 amino acids). Overall, the N-termini of PGC1 β -1 and -2 differ with respect to each other by 26 amino acids (Figure 1D).

Although there is homology between PGC1 α and PGC1 β over the whole molecule, the greatest degree of homology is found at the N- and C-termini. The N-terminus of PGC1 α contains an activation domain, which includes two LXXLL motifs. Human PGC1 β shares between 40 and 50% identity with the first 180 amino acids of PGC1 α . Whereas this region of human PGC1 β contains two LXXLL motifs, in rat and mouse there is an additional LXXLL motif in the N-terminus. The C-terminus of PGC1 α is thought to contribute to this RNA-binding activity of the molecule. There is a high degree of homology (50%) between PGC1 α and PGC1 β in this region, particularly for the PGC1 β -a isoform. In addition to the NR interaction domains, PGC1 β also contains two polyglutamine regions, a serine-rich motif and a putative host cell factor-binding site.

Homology searching using the human PGC1 β cDNA sequence identified a rat clone in the Incyte ZooSeq database that had a high level of homology to PGC1 β . Further sequencing of this clone led to the identification of a full-length cDNA for rat PGC1 β that corresponded to the PGC1 β -2a isoform. At the protein level, the human PGC1 β had 78% identity with the mouse and rat. Mouse and rat shared 93% identity. The RNA recognition motif in the C-terminus is the most conserved domain.

Figure 2 Tissue distribution of PGC1 β

PGC1 β gene expression was analysed in human (A), mouse (B) and rat (C) using a C-terminal RNase protection probe that allows the distinction between the 3' variants PGC1 β -a and -b. Aliquots of 10 μ g of RNA were used to assay for the expression of PGC1 β in these tissues. Sk. m., skeletal muscle; Ep. fat, epididymal fat; Mes. fat, mesenteric fat; RP. fat, retro-peritoneal fat; SC. fat, sub-cutaneous fat; Abd. fat, abdominal fat; m., muscle.

In vivo studies

Tissue distribution of PGC1 β in human, mouse and rats

The tissue distribution of PGC1 β in human, mouse and rat was assessed by RPA. PGC1 β is expressed predominantly in human heart, skeletal muscle and brain (Figure 2A) although it can be detected at low levels in most other tissues, including kidney, pancreas, liver and spleen. By using a probe comprising part of exon 11a, the exon specific to the PGC1 β -a isoform, as well as a region common to both isoforms, we confirmed that the PGC1 β -a isoform is more abundant than PGC1 β -b (Figure 2A) in human. Using a similar approach (a probe spanning exons 1a and 2), we observed that PGC1 β -1 mRNA was the predominantly expressed isoform (results not shown). These results indicate that the most common isoform in human tissues is PGC1 β -1a.

The pattern of expression of PGC1 β in rodents and humans is conserved. Mouse PGC1 β is expressed at high levels in heart, BAT and brain although it can be detected at low levels in most tissues (Figure 2B). Similar to humans, mouse PGC1 β -a is much more abundant than PGC1 β -b (Figure 2B). Rats also share a similar tissue distribution; however they differ in that PGC1 β -a and -b isoforms are expressed at comparable levels (Figure 2C).

In vivo regulation of PGC1 β gene expression in rodents

We explored whether expression of PGC1 β may be regulated *in vivo* by pathophysiological-specific conditions known to affect the expression of PGC1 α . Using the obese Zucker (−/−) and lean (+/−) rat models, we observed that obesity is not associated with changes in PGC1 β mRNA expression or in the relative amount of its specific isoforms (Figure 3A).

Since it was suggested previously that induction of PGC1 β in liver during fasting may be important for hepatic gluconeogenesis [18], we investigated the effects of fasting on PGC1 β mRNA

Figure 3 Regulation of PGC1 β gene expression *in vivo*

(A) Analysis of PGC1 β gene expression in tissues from lean and obese rats using an RPA probe that allows discrimination of the C-terminal isoforms ($n = 2$). WAT, white adipose tissue; m., muscle. (B) Effect of 24 h fasting on PGC1 β gene expression in liver and heart of rats that have been previously on normal or high-fat diet. We illustrate this effect using a probe located at the N-terminus that allows discrimination between the 5' variants PGC1 β -1 and -2 ($n = 3$ animals per condition). (C) Comparison of PGC1 α and PGC1 β gene expression in liver ($n = 4$, per condition) following 24 h fasting/refeeding. The results presented in this Figure were obtained using an RPA probe covering a region common to all isoforms of PGC1 β . (D) Effect of cold exposure [3 h and overnight (ON)] on PGC1 α and PGC1 β mRNA expression in mice detected by RPA. At least two animals per group and condition were analysed. (E) Effect of acute exercise on PGC1 α and PGC1 β gene expression in mouse gastrocnemius muscle. $n = 13$ mice per group and condition (*level of significance, $P < 0.05$).

Figure 4 Cellular localization of PGC1 β

Fluorescence images taken from CHO cells expressing GFP alone or GFP-PGC1 β (green fluorescence). The superposition of the Hoechst coloration and the green fluorescent light in the case of the GFP-PGC1 β construct confirms that PGC1 β is localized into the nucleus.

expression in rodent tissues. Initially, we examined the expression of PGC1 β -1 in heart and liver of Wistar rats either fed or fasted for 17–21 h before tissue collection. We performed this experiment in animals fed previously with either a high-fat (42%) or low-fat diet (2–4%) for 10 weeks. No differences in PGC1 β between fed and fasted rats (Figure 3B) were observed. Given the possibility that differences with previous reports were due to different technical approaches, we performed Northern blots using probes for PGC1 β and PGC1 α . Unlike PGC1 α , the expression of PGC1 β was not up-regulated by fasting in rat liver (results not shown). To determine whether there were species-related differences, we simultaneously measured PGC1 α and PGC1 β mRNA expressions in liver RNA from mice fasted for 24 h (Figure 3C). We confirmed that PGC1 α isoform is markedly induced in liver during fasting, whereas PGC1 β gene expression was unaltered during fasting in mice.

Next we examined the effect of exposure to cold on PGC1 α and PGC1 β expressions. Mice were exposed to cold (4 °C) for 3 h or overnight. As expected, the expression of PGC1 α was up-regulated by exposure to cold in BAT and heart (Figure 3D). Conversely, the expression of PGC1 β in those tissues was not altered by exposure to cold (Figure 3D). Finally, we evaluated whether acute endurance exercise may have any impact on the expression of PGC1 α and PGC2 β genes. We observed that whereas PGC1 α mRNA was induced in exercised mice the expression of PGC1 β was not affected (Figure 3E).

In vitro studies

Cellular localization and co-activation studies

Full-length cDNAs of PGC1 β -1a or -2a were cloned in fusion with the GFP protein and transfected into CHO cells. Whereas cells transfected with GFP control plasmid showed green fluorescence signal in the cytoplasm, cells transfected with PGC1 β -1a/GFP fusion plasmid showed green fluorescence restricted to the nucleus indicative of PGC1 β -1a protein expression (Figure 4). Similar results were obtained for PGC1 β -2a (results not shown).

We tested whether PGC1 β could co-activate TR β 1, PPAR α /RXR α and PPAR γ /RXR α in HepG2 cells and compared the results with PGC1 α . We used either a TR response element or the PPAR response element of the acyl-CoA oxidase gene cloned upstream of the luciferase gene to assess the transactivation efficiency of TR β 1 or PPAR α / γ respectively. PGC1 α and PGC1 β -1a were capable of co-activating TR β 1, leading to a 4-fold increase in transactivation in the presence of T $_3$ at either 100 nM or 1 μ M concentration (Figure 5A). Conversely, PGC1 β -2a did not exert any co-activation effect despite producing a protein, which was targeted to the cell nucleus according to the GFP signal. Co-activation studies of PPAR α (Figure 5B) or PPAR γ (Figure 5C) and their corresponding ligands showed that PGC1 α had a positive effect although less than the one observed with TR β 1 (1.5-fold) and that PGC1 β -1a was a stronger co-activator than PGC1 α (2-fold). Unlike PGC1 α , PGC1 β -1a or -2a did not exert any active transcriptional function in the absence of NRs. To explore whether PGC1 β may co-activate the glucocorticoid receptor we used HeLa cells transfected stably with a GRE- β -galactosidase reporter construct. Co-transfection of these cells with PGC1 β -1a increased the galactosidase response to dexamethasone (Figure 5D).

Ectopic expression of PGC1 β -1a induces mitochondrial biogenesis and increases basal oxygen consumption in L6 myoblasts

Retroviral vectors were utilized to express both mPGC1 α and hPGC1 β -1a in rat L6 myoblasts and investigate the potential role of PGC1 β in regulating mitochondrial biogenesis. TEM on thin sections prepared from these cell lines revealed an obvious proliferation of mitochondria in hPGC1 β -1a cells relative to the empty vector control cells (Figure 6A). An increase in mitochondrial number was also evident in mPGC1 α expressing myoblasts, although to a lesser intensity when compared with the hPGC1 β -1a cells (Figure 6A). The overall mitochondrial morphology and structure did not appear to differ between the various cell lines. The enhanced mitochondrial number is also reflected in a quantitative assessment of cell mitochondrial density (based on calculations from ten randomly selected cells for each myoblast line; performed in duplicate experiments), which indicates clearly that hPGC1 β -1a and mPGC1 α expressions have a 2.7- and 2.0-fold higher cell mitochondrial density respectively when compared with controls (Figure 6B). Moreover, the hPGC1 β -1a and mPGC1 α -induced mitochondrial biogenesis is further supported by the observed >2.0-fold increase in basal oxygen consumption in the respective cell lines relative to non-expressing controls (Figure 6C). The increase in basal oxygen consumption of

Figure 5 Co-activation studies

(A–C) HepG2 cells were transiently co-transfected with FuGENE (Roche) using a ratio of 3 μ l for 1 μ g of DNA. The human pcDNA3-PGC1 β -1a or -2a constructs were used. The mouse pcDNA3-PGC1 α construct was used as a comparison. The pRL-CMV vector (Promega) was co-transfected as a normalized control. Luciferase assays were performed 40 h after transfection using the Dual Luciferase Assay system. In the case of TR β 1, T $_3$ was used at 100 nM or 1 μ M. For PPAR α , the compound GW327647 was used at 100 nM. The RXR α agonist was the compound LG100268 (at 100 nM) and the PPAR γ agonist at 100 nM. These results represent five independent experiments ($n=5$). m, mouse; h, human. (D) GRE 4- β -gal HeLa cells transfected transiently using the calcium phosphate method with a luciferase control plasmid, pGL3 (Promega), to monitor transfection efficiency. pcDNA3 carrier DNA was compared with transfections containing pcDNA3-PGC1 β -1a, pGL3 and carrier pcDNA3. After 2 h, cells were stimulated with a submaximal concentration (1 nM) of dexamethasone (Dex) or left unstimulated. At 48 h post-transfection, the cells were assayed for β -galactosidase and firefly luciferase using a Dual-Light kit (Tropix). These results represent four independent experiments ($n=4$).

Figure 6 Electron microscopy and oxygen consumption studies

(A, B) Ultrastructural analysis using TEM of cells ectopically overexpressing PGC1 α , PGC1 β -1a or empty vector. The percentage of cell volume containing mitochondria was calculated using AnalySis image analysis software (SIS, U.S.A.) in conjunction with a MegaView digital camera on the TEM and a 1000 μ m $\times$ 1000 μ m grid placed over the images. Scale bar, 500 nm. (C) Oxygen consumption in myoblasts expressing mPGC1 α and hPGC1 β -1a. Oxygen consumption rates were measured for myoblasts retrovirally expressing an empty expression vector (white bars), mPGC1 α (black bars) or hPGC1 β -1a (grey bars). Oxygen consumption was measured in the absence of inhibitors (basal) or in the presence of oligomycin and FCCP. Rates were normalized to cell number and measurements made in triplicate. Results are expressed as means $\pm$ S.E.M. These results represent three independent experiments, with two or three repeats of each condition per experiment.

hPGC1 β -1a and mPGC1 α -expressing cells was preserved in the presence of both oligomycin and FCCP (Figure 6C). This implies strongly that increased mitochondrial density and/or electron-transport chain activity is responsible for the observed increase in oxygen consumption.

DISCUSSION

We have cloned the full-length human, mouse and rat PGC1 β /PERC, a homologue of PGC1 α co-activator, and characterized the multiple isoforms *in vivo* and *in vitro*. PGC1 α has received recent attention as a co-ordinator of transcription factors involved in metabolic responses such as adaptive thermogenesis [4], fatty-acid oxidation [8], mitochondrial biogenesis [10], muscle fibre-type-specific differentiation [28] or gluconeogenesis [6] in rodents. Given the importance of PGC1 α as a master integrator of all these complex metabolic programmes, we investigated the potential metabolic roles of this new homologue, PGC1 β .

The human PGC1 β gene, also reported as PERC [19], consists of 13 exons spanning a genomic region of at least 78 kb on chromosome 5. Exon sizes ranged from 37 nt (exon 6) to 1123 nt (exon 5). The human PGC1 β protein shares several motifs with PGC1 α : (i) an RNA recognition motif located at the C-terminus encompassing an RNA-binding region found in proteins having a role in the pre-mRNA maturation and splicing. Such a role has been demonstrated for PGC1 α [22] and is likely to be retained by PGC1 β ; (ii) two polyglutamate tracts found usually in cofactors as proteins rich in polar amino acids can bind to these tracts. Interestingly, neither PGC1 α nor PRC carry such motifs but PERC does [19] and (iii) two LXXLL motifs, necessary for interactions between the co-activators and liganded NRs, also found in PGC1 α . It is worth noting that there are only two LXXLL motifs in the human PGC1 β gene compared with three in the mouse and rat PGC1 β genes and one in the mouse and human PGC1 α gene and this may lead to species-specific functional differences.

PGC1 β , similar to PGC1 α , is expressed predominantly in brown fat, heart, brain and muscle, important metabolic tissues, characterized by their high mitochondrial content. PGC1 β is also expressed at low levels in most tissues. Interestingly, the amount of PGC1 β in a specific tissue appears to be proportional to its mitochondrial content. We have identified several PGC1 β isoforms resulting from the alternative splicing of exons located at the N- and C-termini. The isoform, which we have named PGC1 β -1a, was the most common isoform in humans and mice and corresponded to the isoform identified initially by Lin et al. [18]. The other isoforms were also ubiquitously expressed, although at much lower levels, in humans and mice. However, in rats, the spliced form PGC1 β -2b was expressed at a level comparable with PGC1 β -1a, suggesting that these alternative isoforms may be more relevant in rodent species.

The regulation of PGC1 β and PGC1 α gene expression *in vivo* was markedly different. The PGC1 α gene expression is highly regulated at the transcriptional level. In fact, it has been shown to be markedly induced in specific tissues by cold exposure [4], adrenergic stimulation [29], fasting [5,6,16] or exercise [30,31]. In contrast, we have shown that none of these conditions produced changes in the expression of PGC1 β or changes in the relative expression of its specific isoforms. Our preliminary experiments clearly identify PGC1 α as the only isoform up-regulated in response to exercise, suggesting that, specifically, PGC1 α may be responsible for the muscle mitochondrial oxidative metabolic adaptation to training [32]. As a whole, these results indicate that only PGC1 α appears to be regulated transcriptionally, at least under the current experimental protocols. However, there is still the possibility that, similar to PGC1 α , in the white adipose tissue of the 4E-BP1-knockout mice [33], PGC1 β may be regulated mainly at the translational level. Thus our results show that PGC1 β , like other co-activators, does not seem to be regulated transcriptionally *in vivo*. The lack of transcriptional regulation, together with its relatively widespread tissue

distribution, suggests that PGC1 β may fulfil a more general role, such as maintaining the mitochondria biogenesis necessary to cover basal energy requirements of a tissue. Conversely, PGC1 α , which is regulated transcriptionally by pathophysiological stimuli related to acute increased energy demands, states of high fatty-acid oxidation and gluconeogenesis may be more relevant as a co-ordinator of acute metabolic adaptations to increases in energy demand.

Our studies *in vitro*, in agreement with the PERC studies [19], showed that human PGC1 β isoforms localize to the nucleus. We provide evidence that PGC1 β -1a, the most abundant isoform, co-activates several NRs including the TR, PPAR α and PPAR γ . Also our results indicate that PGC1 β -1a does co-activate the glucocorticoid receptor. The other 5' isoform PGC1 β -2a did not co-activate these transcription factors suggesting the possibility that the N-terminus may include important domains for the co-activation of these transcription factors. This observation is particularly interesting since the main transactivation domains of PGC1 α have been mapped to the N-terminal 120 amino acids [21,34]. Although we have not been able to study other transcription factors, studies from other laboratories have confirmed that the mouse PGC1 β , and specifically the isoform homologous with the human PGC1 β -1a, also co-activates the oestrogen receptor α [19], HNF-4 α and glucocorticoid receptors [18]. Interestingly, Lin et al. [18] showed that mPGC1 β exerts a potent co-activating effect on NRF-1, a key transcription factor involved in mitochondrial biogenesis. Altogether, these results suggest that like PGC1 α , PGC1 β may also have a broad spectrum of actions. Given its predominant expression in tissues that express high levels of PPAR α and mitochondria, it would be expected that the effect of PGC1 β would involve promotion/maintenance of fatty-acid oxidation and mitochondrial biogenesis. Our co-activation studies also showed that PGC1 β potentiates the transcription from the thyroid hormone receptor β 1, an effect that was strong, particularly compared with the other NRs we studied. Thus it is likely that PGC1 β may be an important co-activator integrating some of the actions of thyroid hormone in energy expenditure and mitochondrial biogenesis. The widespread action of thyroid hormones and the relative wide tissue distribution of PGC1 β give support to this possibility.

In an attempt to clarify the function of PGC1 β , we over-expressed PGC1 β in myoblast cells using a retrovirus-mediated gene transfer strategy. The initial characterization of these cells has shown that overexpression of PGC1 β is associated with an increase in the number of mitochondria, which correlated with increased oxygen consumption. The basal rate of oxygen consumption is however a complex function of mitochondrial density, rate of cellular ATP turnover and the rates at which protons are actively pumped across the inner mitochondrial membrane (by the electron-transport chain) and passively leak back across it (e.g. via uncoupling proteins). We eliminated the contribution of ATP turnover to oxygen consumption using oligomycin, which prevents ATP formation by blocking proton translocation through the ATP synthase. Differences in passive proton leak were also removed using the uncoupler FCCP, which dissipates the mitochondrial protonmotive force. Since the approx. 2-fold increase in basal oxygen consumption of hPGC1 β and mPGC1 α -expressing cells was preserved in the presence of both oligomycin and FCCP, this implies strongly that increased mitochondrial density and/or electron-transport chain activity is responsible for the observed increase in oxygen consumption, corroborating the TEM results. These results suggest that like PGC1 α , PGC1 β may also play a role in mitochondrial biogenesis. Thus it is likely that PGC1 α and PGC1 β may represent complementary regulatory mechanisms of

a mitochondrial biogenesis programme. Based on these results, we hypothesized that whereas PGC1 α may co-ordinate the response to the energy demands generated during acute metabolic situations (e.g. cold, hypoglycaemia), PGC1 β may act more as a co-ordinator to promote the mitochondrial biogenesis necessary to meet the basal requirements of a specific tissue. This process may take place by co-activating the transcriptions factors involved in the basal mitochondrial biogenesis, and involving probably the programme of thyroid hormone action.

In summary, we have identified and characterized the human PGC1 β /PERC cDNA and genomic sequences including several minor alternative splice isoforms. The latter might be more relevant functionally in rodents. The tissue distribution of PGC1 β mRNA is conserved among species and, contrary to PGC1 α , does not seem to be regulated transcriptionally *in vivo* raising questions about the role of PGC1 β in adaptive thermogenesis or gluconeogenesis. Finally, we provide evidence that over-expression of PGC1 β is associated with increased number of mitochondria and oxygen consumption, suggesting that PGC1 β may play a role in constitutive non-adrenergic-mediated mitochondrial biogenesis. Future experiments will characterize, in detail, the metabolic characteristics and molecular mechanisms modulating mitochondrial biogenesis and fatty-acid metabolism in these cell lines.

We thank Catrin Williams, Alex Kvist and Quentin Descat for excellent technical assistance. Research in A.V.-P.'s and S.O.R.'s laboratories was funded by Wellcome Trust. A.M. was supported by a Marie Curie Fellowship and V.C. by a Wellcome Trust fellowship. C.L. and K.G. were funded by MRC fellowships, and S.S. was funded by Deutsche Forschungsgemeinschaft (DFG).

REFERENCES

- Naar, A. M., Lemon, B. D. and Tjian, R. (2001) Transcriptional coactivator complexes. *Annu. Rev. Biochem.* **70**, 475–501
- Lee, K. C. and Lee, K. W. (2001) Nuclear receptors, coactivators and chromatin: new approaches, new insights. *Trends Endocrinol. Metab.* **12**, 191–197
- Tontonoz, P., Hu, E. and Spiegelman, B. M. (1994) Stimulation of adipogenesis in fibroblasts by PPAR γ 2, a lipid-activated transcription factor. *Cell (Cambridge, Mass.)* **79**, 1147–1156
- 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
- Yoon, J. C., Puigserver, P., Chen, G., Donovan, J., Wu, Z., Rhee, J., Adelman, 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
- 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
- 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
- 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
- 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
- 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
- Michael, L. F., Wu, Z., Cheatham, R. B., Puigserver, P., Adelman, 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
- Knutti, D. and Kralli, A. (2001) PGC-1, a versatile coactivator. *Trends Endocrinol. Metab.* **12**, 360–365
- Hermanson, O., Glass, C. K. and Rosenfeld, M. G. (2002) Nuclear receptor coregulators: multiple modes of modification. *Trends Endocrinol. Metab.* **13**, 55–60

- 14 Hagen, T. and Vidal-Puig, A. (2002) Mitochondrial uncoupling proteins in human physiology and disease. *Minerva Med.* **93**, 41–57
- 15 Crowley, V. and Vidal-Puig, A. J. (2001) Mitochondrial uncoupling proteins (UCPs) and obesity. *Nutr. Metab. Cardiovasc. Dis.* **11**, 70–75
- 16 Lehman, J. J., Barger, P. M., Kovacs, A., Saffitz, J. E., Medeiros, D. M. and Kelly, D. P. (2000) Peroxisome proliferator-activated receptor γ coactivator-1 promotes cardiac mitochondrial biogenesis. *J. Clin. Invest.* **106**, 847–856
- 17 Lehman, J. J. and Kelly, D. P. (2002) Transcriptional activation of energy metabolic switches in the developing and hypertrophied heart. *Clin. Exp. Pharmacol. Physiol.* **29**, 339–345
- 18 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
- 19 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
- 20 Andersson, U. and Scarpulla, R. C. (2001) Pgc-1-related coactivator, a novel, serum-inducible coactivator of nuclear respiratory factor 1-dependent transcription in mammalian cells. *Mol. Cell Biol.* **21**, 3738–3749
- 21 Scarpulla, R. C. (2002) Transcriptional activators and coactivators in the nuclear control of mitochondrial function in mammalian cells. *Gene* **286**, 81–89
- 22 Monsalve, M., Wu, Z., Adelmant, G., Puigserver, P., Fan, M. and Spiegelman, B. M. (2000) Direct coupling of transcription and mRNA processing through the thermogenic coactivator PGC-1. *Mol. Cell* **6**, 307–316
- 23 Vidal-Puig, A., Jimenez-Linan, M., Lowell, B. B., Hamann, A., Hu, E., Spiegelman, B., Flier, J. S. and Moller, D. E. (1996) Regulation of PPAR γ gene expression by nutrition and obesity in rodents. *J. Clin. Invest.* **97**, 2553–2561
- 24 Vidal-Puig, A. J., Considine, R. V., Jimenez-Linan, M., Werman, A., Pories, W. J., Caro, J. F. and Flier, J. S. (1997) Peroxisome proliferator-activated receptor gene expression in human tissues. Effects of obesity, weight loss, and regulation by insulin and glucocorticoids. *J. Clin. Invest.* **99**, 2416–2422
- 25 Jakubowski, M. (1995) In *Methods in Neuroscience*, vol 25: Receptor Molecular Biology (Sealfon, S.C., ed.), pp. 470–491, Academic Press, San Diego
- 26 Bright, N. A., Reaves, B. J., Mullock, B. M. and Luzio, J. P. (1997) Dense core lysosomes can fuse with late endosomes and are re-formed from the resultant hybrid organelles. *J. Cell Sci.* **110**, 2027–2040
- 27 Griffiths, G. (1993) *Fine Structure Immunocytochemistry*, Springer-Verlag, Berlin
- 28 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
- 29 Boss, O., Bachman, E., Vidal-Puig, A., Zhang, C. Y., Peroni, O. and Lowell, B. B. (1999) Role of the β (3)-adrenergic receptor and/or a putative β (4)-adrenergic receptor on the expression of uncoupling proteins and peroxisome proliferator-activated receptor- γ coactivator-1. *Biochem. Biophys. Res. Commun.* **261**, 870–876
- 30 Terada, S., Goto, M., Kato, M., Kawanaka, K., Shimokawa, T. and Tabata, I. (2002) Effects of low-intensity prolonged exercise on PGC-1 mRNA expression in rat epitrochlearis muscle. *Biochem. Biophys. Res. Commun.* **296**, 350
- 31 Wu, H., Kanatous, S. B., Thurmond, F. A., Gallardo, T., Isotani, E., Bassel-Duby, R. and Williams, R. S. (2002) Regulation of mitochondrial biogenesis in skeletal muscle by CaMK. *Science* **296**, 349–352
- 32 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
- 33 Tsukiyama-Kohara, K., Poulin, F., Kohara, M., DeMaria, C. T., Cheng, A., Wu, Z., Gingras, A. C., Katsume, A., Elchebly, M., Spiegelman, B. M. et al. (2001) Adipose tissue reduction in mice lacking the translational inhibitor 4E-BP1. *Nat. Med.* **7**, 1128–1132
- 34 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

Received 3 February 2003/26 March 2003; accepted 4 April 2003

Published as BJ Immediate Publication 4 April 2003, DOI 10.1042/BJ20030200