ACCELERATED PUBLICATION

Hepatocyte nuclear factor-4 α mediates the stimulatory effect of peroxisome proliferator-activated receptor γ co-activator-1 α (PGC-1 α) on glucose-6-phosphatase catalytic subunit gene transcription in H4IIE cells

Jared N. BOUSTEAD, Beth T. STADELMAIER, Angela M. EEDS, Peter O. WIEBE, Christina A. SVITEK, James K. OESER and Richard M. O'BRIEN¹

Department of Molecular Physiology and Biophysics, Vanderbilt University Medical School, Nashville, Tennessee 37232, U.S.A.

It has recently been shown that adenoviral-mediated expression of peroxisome proliferator-activated receptor γ co-activator-1 α (PGC-1 α) in hepatocytes stimulates glucose-6-phosphatase catalytic subunit (G6Pase) gene expression. A combination of fusion gene, gel retardation and chromatin immunoprecipitation assays revealed that, in H4IIE cells, PGC-1 α mediates this

stimulation through an evolutionarily conserved region of the G6Pase promoter that binds hepatocyte nuclear factor-4 α .

Key words: diabetes, gluconeogenesis, HNF-4, gluconeogenesis, phosphoenolpyruvate carboxykinase.

INTRODUCTION

Glucose-6-phosphatase catalyses the final step of the gluconeogenic pathway, the hydrolysis of glucose-6-phosphate to glucose and inorganic phosphate, and activity of the enzyme is predominantly detected in liver and kidney [1,2]. The enzyme is located in the endoplasmic reticulum membrane and is thought to exist as a multi-component system, consisting of a catalytic subunit in addition to specific transporters for glucose 6-phosphate, glucose and inorganic phosphate [1,2]. Mutations that inactivate the glucose-6-phosphatase catalytic subunit (G6Pase) give rise to glycogen storage disease type 1a [3], whereas overexpression of G6Pase is thought to contribute to the increased hepatic glucose production characteristic of both type I and type II diabetes [4].

Peroxisome proliferator-activated receptor γ co-activator (PGC)-1 α was originally identified through its interaction with peroxisome proliferator-activated receptor (PPAR) γ [5], but it has subsequently been shown to interact with multiple members of the nuclear hormone receptor superfamily, as well as other transcription factors [6]. PGC-1 α appears to be an important regulator of mitochondrial biogenesis, respiration and thermogenesis [reviewed in [6]], as well as hepatic gluconeogenesis [7,8]. Recently, two PGC-1 α homologues have been identified, PGC-1 β [9] and PGC-1-related co-activator ('PRC') [10]. In liver, expression of both PGC-1 α and PGC-1 β are markedly induced by fasting [9], and adenoviral-mediated expression of PGC-1 α in primary hepatocytes induces G6Pase and phosphoenolpyruvate carboxykinase (PEPCK) gene expression, as well as glucose production [7]. In the present study, we describe the use of a combination of fusion gene, gel retardation and chromatin immunoprecipitation assays to identify hepatocyte nuclear factor-4 α (HNF-4 α) as the transcription factor that mediates the induction of mouse G6Pase gene expression by PGC-1 α in H4IIE cells.

MATERIALS AND METHODS

Materials

[α -³²P]dATP (> 3000 Ci mmol⁻¹) was obtained from NEN Life Sciences (MA, U.S.A.). Specific antisera to HNF-4 (sc-6556 and sc-8987), PPAR α (sc-9000), PPAR γ (sc-7196) and rabbit IgG (sc-2027) were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, U.S.A.).

Plasmids

The generation of mouse G6Pase-chloramphenicol acetyltransferase fusion genes, containing promoter sequence located between -484/+66, -271/+66 and -158/+66, relative to the transcription start site, has been previously described [11]. These promoter fragments were all sub-cloned into the pGL3 MOD vector. This vector is based on the pGL3 Basic firefly luciferase vector (Promega, Madison, WI, U.S.A.) but contains a modified polylinker [12]. Additional G6Pase promoter fragments containing sequence between -297/+66, -85/+66 and -35/+66 were generated using restriction enzyme digestion or PCR and were also cloned into the pGL3 MOD vector. Promoter fragments generated by PCR were completely sequenced to ensure the absence of polymerase errors.

Expression vectors encoding PPAR γ and retinoid X receptor (RXR) α were gifts from Dr Rajnish A. Gupta and Raymond N. DuBois (both at Vanderbilt University Medical Center, Nashville, TN, U.S.A.). An expression vector, designated pBJ5mHNF-1 [13], that contains a mouse HNF-1 α cDNA in the pBJ5 vector was a gift from Dr Gerald R. Crabtree (Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, CA, U.S.A.). The pBJ5 vector was regenerated by removing the HNF-1 α cDNA as an *Eco*RI fragment. A mouse PGC-1 α cDNA in the pME18S vector (I.M.A.G.E. clone #1972766) was purchased from the American

Abbreviations used: ChIP, chromatin immunoprecipitation; DTT, dithiothreitol; G6Pase, glucose-6-phosphatase catalytic subunit; HNF, hepatocyte nuclear factor; MODY, maturity onset diabetes of the young; PEPCK, phosphoenolpyruvate carboxykinase; PGC, PPAR γ co-activator; PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X receptor; TBE, Tris/borate/EDTA buffer.

¹ To whom correspondence should be addressed (e-mail richard.obrien@mcmail.vanderbilt.edu).

Type Culture Collection, Manassas, VA, U.S.A. This cDNA clone appears to represent a splice variant since it contains the entire 797 amino acid PGC-1 α open reading frame [5] minus amino acids 476–487. This PGC-1 α cDNA sequence was isolated from the pME18S vector as an *Xho*I fragment and was ligated into the *Xho*I site of pBJ5.

Cell culture, transient transfections and luciferase assays

Rat H4IIE hepatoma cells were cultured in Dulbecco's modified Eagle's medium containing 2.5% (v/v) newborn calf serum and 2.5% (v/v) fetal calf serum and were transiently transfected in solution using the calcium phosphate-DNA co-precipitation method, as previously described [11]. Where indicated, expression vectors encoding renilla luciferase (0.15 μ g), PGC-1 α or the empty pBJ5 vector control (2.5 μ g), were co-transfected with the reporter gene construct (15 μ g). Cells were harvested and luciferase assays were performed using Promega's Dual Luciferase Reporter Assay System, as previously described [14].

Nuclear extract preparation, *in vitro* coupled transcription and translation and gel retardation assays

H4IIE nuclear extract was prepared by the method of Andrews and Faller [15], except that cells were lysed using NP-40 to isolate nuclei [16]. Briefly, cells were lysed in 10 mM Tris/HCl (pH 7.4), 10 mM NaCl, 3 mM MgCl₂, 0.5% NP-40, 0.5 mM dithiothreitol (DTT), 0.5 mM PMSF and 1 $\times$ Protease Inhibitor Cocktail (Roche) and the isolated nuclei were extracted using 20 mM Hepes (pH 7.9), 0.4 M NaCl, 0.75 mM spermidine, 0.15 mM spermine, 0.2 mM EDTA, 2 mM EGTA, 25% glycerol, 2 mM DTT and 1 mM PMSF. PPAR γ and RXR α were generated using Promega's TNT T7 Coupled Reticulocyte Lysate System according to the manufacturer's instructions. Oligonucleotides representing the sense and antisense wild-type G6Pase promoter sequence between –82 and –47 (see Figure 2A) were synthesized with *Bam*HI compatible ends and subsequently gel purified, annealed, and labelled with [α -³²P]ATP using the Klenow fragment of *Escherichia coli* DNA Polymerase I to a specific activity of approx. 2.5 μ Ci/pmol [17]. The labelled double-stranded oligonucleotide probe (approx. 7 fmol, 50000 c.p.m.) was incubated with either (i) H4IIE nuclear extract (2 μ g) in a final reaction volume of 20 μ l containing 20 mM Hepes (pH 7.8), 80 mM NaCl, 50 mM MgCl₂, 0.38 mM spermidine, 0.08 mM spermine, 0.1 mM EDTA, 1 mM EGTA, 2 mM DTT, 12.5% (v/v) glycerol and 1 μ g of poly(dI-dC) · poly(dI-dC) or (ii) with reticulocyte lysate reactions (1 μ l) in a final reaction volume of 20 μ l containing 19 mM Hepes (pH 7.8), 52 mM NaCl, 1 mM DTT, 9.5% (v/v) glycerol and 1 μ g of poly(dI-dC) · poly(dI-dC). After incubation for 10 min at room temperature, the reactants were loaded on to a 6% polyacrylamide gel containing 0.5 $\times$ Tris/borate/EDTA buffer (TBE) and 2.5% glycerol and then electrophoresed at room temperature for 120 min at 150 V in 0.5 $\times$ TBE (1 $\times$ TBE is 89 mM Tris, 89 mM boric acid and 2 mM EDTA). Following electrophoresis, gels were dried, exposed to Kodak XAR5 film, and binding was analysed by autoradiography. For supershift experiments, specific antisera (1 μ l) were added, as indicated, to the binding reaction and the incubation was continued for an additional 15 min at room temperature.

Western blotting

To demonstrate the specificity of the HNF-4 antisera, 30 μ g of H4IIE nuclear extract was electrophoresed on a 7.5% SDS/

PAGE gel and the proteins were transferred to PVDF, as previously described [18]. Immunoblot analysis was then performed using an HNF-4 antiserum as the primary antibody and a goat anti-rabbit IgG antiserum conjugated to horseradish peroxidase (Calbiochem) as the secondary antibody. Antibody binding was detected using the SuperSignal West Femto kit (Pierce) according to the manufacturer's instructions.

Chromatin immunoprecipitation (ChIP) assays

Chromatin immunoprecipitation assays were performed using a previously described modification [19] of published procedures [20,21]. Fragmented chromatin was immunoprecipitated using either HNF-4 antiserum (sc-8987; Santa Cruz) or rabbit IgG (sc-2027; Santa Cruz). PCR primers were designed to amplify the G6Pase promoter [5'-(–162) CGTAAATCACCTGAAC-ATGTTTGCATCAACC (–131)-3' and 5'-(–17) CCTTGC-CCATTTTATATGCCCTGGATTC (–44)-3'] and exon 5 [5'-(+723) GTTTGGTTTCGCACTTGGAT (+742)-3' and 5'-(+940) GCAGTTCTCCTTTCAGCTC (+921)-3']. The products were visualized by electrophoresis on 2% agarose gels containing ethidium bromide.

RESULTS

Deletion of the mouse G6Pase promoter sequence between –85 and –36 abolishes the stimulatory effect of PGC-1 α on basal G6Pase fusion gene expression

Spiegelman and co-workers have shown that adenoviral-mediated expression of PGC-1 α in primary hepatocytes induces G6Pase gene expression [7]. To identify the G6Pase promoter region that mediates the stimulatory effect of PGC-1 α on basal G6Pase gene expression, H4IIE cells were co-transfected with various 5' truncated G6Pase-luciferase fusion genes and either a control expression vector or the same vector encoding PGC-1 α . Similar to results previously reported using HepG2 cells [11], basal G6Pase fusion gene expression in H4IIE cells decreased markedly when the promoter region between –484 and –272 was deleted but, in contrast to HepG2 cells [22], further deletion of the region located between –271 and –36 resulted in minimal change (Figure 1A). The induction of G6Pase fusion gene expression by PGC-1 α , expressed as percent of maximal induction (Figure 1B) or fold induction (Figure 1C), decreased when the promoter region located between –484 and –298 was deleted. However, this may be accounted for by the decreased basal promoter activity (Figure 1A). In contrast, the induction of G6Pase fusion gene expression by PGC-1 α was abolished when the promoter region located between –85 and –36 was deleted (Figures 1B and 1C), but this deletion was associated with only a minor change in basal promoter activity (Figure 1A). This result suggests that the induction of G6Pase fusion gene expression by PGC-1 α requires an element located between –85 and –36.

Mutation of an HNF-4 and PPAR/RXR binding site in the G6Pase promoter abolishes the stimulatory effect of PGC-1 α on basal G6Pase fusion gene expression

Examination of the residues between –85 and –36 revealed a potential binding site, located between –76 and –64, for HNF-4 and the PPAR/RXR heterodimer (Figure 2A). This binding site is conserved in the equivalent location within both the human and rat G6Pase promoters [23,24] and PGC-1 α has previously been shown to interact with PPAR α , PPAR γ and HNF-4 [5–7, 25].

Figure 1 A G6Pase promoter element located in the region between -85 and -35 mediates the response to PGC-1 α

H4IIE cells were transiently co-transfected, as described in the Materials and methods section, with a series of 5'-truncated G6Pase-luciferase fusion genes (15 μ g), and an expression vector encoding renilla luciferase (0.15 μ g), and also, either a pBJ5 expression vector encoding PGC-1 α or the empty pBJ5 vector control (2.5 μ g). Following transfection, cells were incubated for 18–20 h in serum-free medium. Cells were then harvested and luciferase activity assayed as described in the Materials and methods section. Results are presented as the ratio of firefly: renilla luciferase activity and are expressed as a percentage of that obtained with the -484 G6Pase-luciferase fusion gene in the presence of the empty vector control (A) or the PGC-1 α expression vector (B). In (C) results are presented as the ratio of firefly luciferase activity, corrected for renilla luciferase in the cell lysate, in PGC-1 α transfected versus empty vector transfected cells and are expressed as fold induction. Results represent the mean $\pm$ S.E.M. of 3 experiments using independent preparations of all fusion gene constructs in which each experimental condition was assayed in duplicate.

To assess the role of this HNF-4/PPAR/RXR binding site in mediating the stimulatory effect of PGC-1 α on G6Pase fusion gene expression, this element was mutated by site-directed mutagenesis within the context of the -85 to $+66$ G6Pase promoter fragment (Figure 2A). The construct generated, designated -85 HNF-4 SDM, was analysed by transient transfection of H4IIE cells (Figure 2B). Mutation of this HNF-4/PPAR/RXR motif abolished the stimulation of G6Pase-luciferase fusion gene expression by PGC-1 α (Figure 2B). The effect of this mutation was equivalent to that seen when the entire -85 to -36 promoter region was deleted, as is the case with the -35 G6Pase-luciferase fusion gene (Figure 2B). This suggests that HNF-4 and/or PPAR/RXR may be the factors that are mediating the stimulatory effect of PGC-1 α on G6Pase-luciferase fusion gene expression through this promoter region.

Figure 2 Mutation of the G6Pase promoter region between -76 and -64 abolishes the response to PGC-1 α

(A) Comparison of the wild-type and mutated mouse G6Pase promoter sequence located between -82 and -47 . The potential HNF-4 and PPAR/RXR binding site is boxed and the consensus sequence was taken from [48]. (B) H4IIE cells were transiently co-transfected, as described in the Materials and methods section, with various G6Pase-luciferase fusion genes (15 μ g) and expression vectors encoding renilla luciferase (0.15 μ g) and PGC-1 α (2.5 μ g). The G6Pase-luciferase fusion genes contained either distinct lengths of wild-type (WT) promoter sequence, as indicated by the 5' deletion end-points, or a site-directed mutation of the HNF-4 site, designated -85 HNF-4 SDM. Following transfection, cells were incubated for 18–20 h in serum-free medium. Cells were then harvested and luciferase activity assayed as described in the Materials and methods section. Results are presented as the ratio of firefly: renilla luciferase activity and are expressed as a percentage of that obtained with the -85 G6Pase-luciferase fusion gene. Results represent the mean $\pm$ S.E.M. of 3 experiments using independent preparations of all fusion gene constructs in which each experimental condition was assayed in duplicate.

HNF-4 α and PPAR γ /RXR α bind to the G6Pase promoter *in vitro*

To provide evidence that HNF-4 and/or PPAR/RXR are indeed the factors that are mediating the stimulatory action of PGC-1 α on G6Pase fusion gene expression, protein binding to the -82 to -47 G6Pase promoter region was analysed using the gel retardation assay. When a labelled double-stranded oligonucleotide, designated G6P WT, representing this sequence was incubated with nuclear extract prepared from H4IIE cells, a single major protein–DNA complex was detected (Figure 3A). Competition experiments, in which a 100-fold molar excess of unlabelled DNA was included with the labelled probe, were used to correlate protein binding with the PGC-1 α response. The G6P WT oligonucleotide competed effectively for formation of the protein–DNA complex (Figure 3A). By contrast, an oligonucleotide, designated G6P MUT, that contains a mutation identical to that described in the -85 HNF-4 SDM construct (Figure 2B) failed to compete with the labelled probe for protein binding (Figure 3A). Thus, this protein–DNA complex represents specific protein binding and its formation correlates with the PGC-1 α response.

To identify the factor present in this specific protein–DNA complex, a gel retardation assay was performed in which two distinct antisera specific for HNF-4 were added to the binding

Figure 3 HNF-4 α and PPAR γ /RXR α bind the G6Pase promoter region between -82 and -47 *in vitro*

(A) A labelled double-stranded oligonucleotide representing the wild-type mouse G6Pase promoter region between -82 and -47 was incubated in the absence (—) or presence of a 100-fold molar excess of the unlabelled oligonucleotides shown, representing either the wild-type (WT) or mutated (MUT) -82 to -47 sequence (Figure 2A). H4IIE nuclear extract was then added. (B) H4IIE cell nuclear extract was incubated with the labelled probe described above in the absence (—) or presence of the indicated antisera. (C) Reticulocyte lysate was programmed as indicated. (D) Reticulocyte lysate programmed with both PPAR γ and RXR α was incubated with the labelled probe described above in the absence (—) or presence of the indicated antisera. In (A)–(D) protein binding was analysed using the gel retardation assay as described in the Materials and Methods section. In (C) and (D) the representative autoradiographs show only the retarded complex and not the free probe, which was present in excess. (E) HNF-4 α expression in H4IIE cells was analysed by immunoblot analysis as described in the Materials and methods section.

reaction (Figure 3B). One of these antisera (sc-6556) is specific for HNF-4 α whereas the other (sc-8987) preferentially binds HNF-4 α over HNF-4 γ . While HNF-4 α and HNF-4 γ are expressed in multiple tissues, only HNF-4 α is expressed in liver [26]. Both HNF-4 antisera reduced the migration of the specific protein–DNA interaction resulting in the formation of a ‘supershifted’ complex (Figure 3B). In contrast, antisera specific for PPAR α and PPAR γ had no effect on the formation of the specific protein–DNA complex (Figure 3B).

The absence of detectable PPAR binding using H4IIE cell nuclear extract is consistent with the very low expression of both PPAR α and PPAR γ in hepatoma cells [27,28]. However, the possibility that either PPAR α or PPAR γ could also mediate the action of PGC-1 on G6Pase gene expression *in vivo* cannot be dismissed. To determine whether PPAR γ /RXR α heterodimers can bind the -82 to -47 G6Pase promoter region, these

Figure 4 The G6Pase promoter binds HNF-4 α *in situ*

HNF-4 α binding to the G6Pase promoter was analysed *in situ* using the ChIP assay. Chromatin from formaldehyde-treated H4IIE cells was immunoprecipitated using anti-HNF-4 antiserum or, as a control, using IgG. The presence of the G6Pase promoter (A) and exon 5 (B) in the chromatin preparation prior to immunoprecipitation (1:100–1000 input) and in the immunoprecipitates was then assayed using PCR as described in the Materials and methods section. MW, molecular weight markers.

proteins were generated using an *in vitro* reticulocyte lysate expression system and protein binding was analysed using the gel retardation assay. When the labelled G6P WT oligonucleotide representing this sequence was incubated with unprogrammed lysate, or lysate programmed with PPAR γ or RXR α individually, no protein–DNA complex was detected (Figure 3C). In contrast, when the labelled G6P WT probe was incubated with lysate programmed with both PPAR γ and RXR α together, a single protein–DNA complex was detected (Figure 3C). Competition experiments, as described above for HNF-4, demonstrated that the formation of this PPAR γ /RXR α -DNA complex correlates with the PGC-1 response (results not shown). Figure 3(D) shows that neither HNF-4 antisera reduced the migration of the PPAR γ /RXR α -DNA complex, whereas addition of an antiserum specific for PPAR γ , though not PPAR α , resulted in the formation of a ‘supershifted’ complex. This experiment demonstrates the specificity of the HNF-4 antisera consistent with the observation that HNF-4 antiserum sc-8987 (Figure 3E) and sc-6556 (results not shown) both recognize a protein of approx. 52 kDa in Western blot analysis, which matches the size of rat HNF-4 α . Both antisera also recognize a second lower molecular mass species, suggesting that this may represent proteolysed HNF-4 α (Figure 3E and results not shown). The size of this lower molecular mass species is inconsistent with rat PPAR α (approx. 52 kDa) and rat PPAR γ (approx. 54 kDa). Thus, the combination of these protein binding experiments and the functional data shown in Figure 2(B) are consistent with the hypothesis that, in H4IIE cells, HNF-4 α mediates the stimulatory action of PGC-1 α on G6Pase gene expression.

HNF-4 α binds to the G6Pase promoter *in situ*

ChIP assays [20,29] were performed to assess HNF-4 α binding to the G6Pase promoter within intact cells. This assay is important for confirming that interactions that occur *in vitro* also occur *in situ*. Fragmented chromatin from formaldehyde cross-linked H4IIE cells was subjected to immunoprecipitation with HNF-4 antiserum, or with IgG as a control, and the presence of the G6Pase promoter in the immunoprecipitates was then analysed by PCR using primers that recognize the G6Pase gene sequence between -162 and -17. As can be seen from Figure 4(A), the G6Pase promoter was enriched in the HNF-4-immunoprecipitate compared with the IgG control. To test the specificity of this

antibody–protein interaction, these immunoprecipitates were also analysed for the presence of exon 5 of the rat G6Pase gene [24] using PCR primers that recognize the G6Pase gene sequence between +723 and +940. This genomic region would not be predicted to associate with HNF-4. As expected, no enrichment of G6Pase exon 5 was detected in the HNF-4-immunoprecipitate compared with the IgG control (Figure 4B). These results suggest that HNF-4 α binds to the G6Pase promoter inside intact cells.

DISCUSSION

Adenoviral-mediated expression of PGC-1 α in primary hepatocytes stimulates G6Pase gene expression [7]. A combination of fusion gene (Figures 1 and 2), gel retardation (Figure 3) and ChIP assays (Figure 4) suggest that, in H4IIE cells, PGC-1 α mediates this stimulation through an evolutionarily conserved region of the G6Pase promoter, located between –76 and –64 in the mouse gene, that binds HNF-4 α . Although a PPAR γ /RXR α heterodimer can also bind this element *in vitro* (Figures 3C and 3D), PPAR α and PPAR γ binding to this element cannot be detected using H4IIE nuclear extracts (Figure 3B), probably because expression of both factors is very low in hepatoma cells [27,28]. However, since both PPAR γ and PPAR α are expressed in liver *in vivo* [30], the possibility exists that both of these factors, in addition to HNF-4 α , may be important for the induction of G6Pase expression by PGC-1 α *in vivo*. Future studies examining the effect of PGC-1 on G6Pase gene expression in mice containing liver-selective deletions of the genes encoding PPAR α , PPAR γ or HNF-4 α should be able to address this issue.

Since HNF-4 α also mediates the stimulatory effect of PGC-1 α on PEPCK [7] and carnitine palmitoyltransferase I [31] gene transcription, this suggests that it may co-ordinate the induction of liver gene expression by PGC-1 α . Moreover, since both HNF-4 α [32,33] and PGC-1 α [5,34,35] are also expressed in kidney and pancreas, it is possible that HNF-4 α is important for PGC-1 α action in those tissues. Interestingly, heterozygous mutations in the HNF-4 α gene give rise to maturity onset diabetes of the young (MODY) type 1 [36] and recent data have established a potential connection between genetic variation in the PGC-1 α gene and the pathogenesis of type II diabetes [37]. MODY1 patients exhibit defective insulin secretion with minimal changes in liver function [36], but whether this defective insulin secretion can be explained, in part, by loss of PGC-1 α action in the pancreas remains to be determined.

It is surprising that the presence of one wild-type HNF-4 α allele is sufficient for maintaining normal liver function in MODY1 patients given the critical roles of HNF-4 α in liver development [38] and in the maintenance of hepatic gene expression [39]. However, a similar phenotype is also seen in MODY3 patients who have heterozygous mutations in the HNF-1 α gene [36]. As with MODY1, MODY3 patients exhibit defective insulin secretion with minimal changes in liver function [36], again despite the critical role of HNF-1 α in the maintenance of hepatic gene expression [40]. These observations are particularly surprising given that HNF-4 α regulates the hepatic expression of HNF-1 α [41] and HNF-1 α regulates the hepatic transactivation potential of HNF-4 α [40]. Thus, disruption of HNF-1 α gene expression also affects HNF-4 α -regulated gene expression and vice versa. Moreover, there are several genes, such as G6Pase (Figures 1–4 and [42,43]), whose expression are regulated by both factors and it would have been predicted that the hepatic expression of such genes should have been significantly affected in MODY1 and MODY3.

Although PGC-1 α has been suggested to be a key regulator of hepatic gluconeogenesis [7,8] the relative importance of PGC-1 α

in the regulation of gluconeogenesis remains to be established. Thus, while Yoon et al. [7] showed that PGC-1 α mRNA and PEPCK mRNA were induced by fasting with a similar time course in mouse liver, PGC-1 α protein was not measured. Moreover, an analysis of cAMP- and glucagon-stimulated gene expression in rat primary hepatocytes at earlier time points shows that PEPCK mRNA is actually induced before PGC-1 α mRNA [31]. Similarly, in serum-starved H4IIE cells, in which PGC-1 α and PGC-1 β expression should be very low [5,9], cAMP and glucocorticoids rapidly induce PEPCK gene transcription, effects that are not blocked by protein synthesis inhibitors ([44] and D. K. Granner, personal communication). One of the major difficulties in interpreting the physiological significance of studies on PEPCK gene expression arises because different results have been obtained in H4IIE cells, HepG2 cells and in primary hepatocytes [7,8,45,46]. For example, Herzig et al. [8] proposed that the cAMP-induced expression of PGC-1 α mediates the activation of the gluconeogenic programme in response to glucocorticoid signals on the basis of the observation that dexamethasone and PGC-1 α together induce PEPCK fusion gene expression in HepG2 cells, whereas either agent alone has no effect. However, in H4IIE cells, dexamethasone and PGC-1 α independently induce PEPCK fusion gene expression [7]. Overall, these results are consistent with a model in which PGC-1 α acts to potentiate, but is not required for cAMP- and glucocorticoid-regulated PEPCK gene expression. Finally, it is apparent that, except under conditions of prolonged fasting, the regulation of hepatic gluconeogenesis by insulin and glucagon is rapid and independent of changes in gene expression [47].

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