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Peroxisome proliferator-activated receptor γ coactivator family members competitively regulate hepatitis b virus biosynthesis

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ABSTRACT

Transcriptional coactivators represent critical components of the transcriptional pre-initiation complex and are required for efficient gene activation. Members of the peroxisome proliferator-activated receptor gamma coactivator 1 (PGC1) family differentially regulate hepatitis b virus (HBV) biosynthesis. Whereas PGC1α has been shown to be a potent activator of HBV biosynthesis, PGC1β only very poorly activates HBV RNA and DNA synthesis in human hepatoma (HepG2) and embryonic kidney (HEK293T) cells. Furthermore, PGC1β inhibits PGC1α-mediated HBV biosynthesis. These observations suggest that a potential competition between human hepatoma (HepG2) and embryonic kidney (HEK293T) cells PGC1α and PGC1β for common transcription factor target(s) may regulate HBV transcription and replication in a context and signal transduction pathway dependent manner.

1. Introduction

Hepatitis B virus (HBV) replicates in the hepatocytes of humans and higher primates by reverse transcription of the viral pregenomic 3.5-kbp RNA intermediate (Hu et al., 2000; Lanford et al., 1998; Raney and McLachlan, 1991; Seeger and Mason, 2000; Summers and Mason, 1982; Warren et al., 1999). The level of pregenomic RNA, and hence viral replication intermediates, are governed by liver-enriched transcription factors including nuclear receptors which also regulate hepatic metabolic pathways in response to various metabolic cues (Reese et al., 2011b, 2013; Tang and McLachlan, 2001). Nuclear receptor-dependent activation of their target genes requires the recruitment of transcriptional coactivators to promote the modulation of the local chromatin environment and also RNA polymerase II plus the general transcription factors necessary for initiation of RNA synthesis (Aoyagi and Archer, 2008; Barrero and Malik, 2006; Wallberg et al., 2003). *In vivo* studies have indicated that transcription of HBV genomic DNA occurs in the context of chromatin which may become transcriptionally active after the necessary covalent modification of histones and remodeling of nucleosomes (Bock et al., 1994; Newbold et al., 1995; Tropberger et al., 2015). Consequently, an understanding of the steps governing the selective recruitment of specific coactivators to viral promoters and their relative roles in regulating HBV transcription appears crucial to understand the regulation of viral biosynthesis.

Coactivators are recruited to enhancer and promoter transcriptional regulatory elements by sequence-specific DNA binding proteins, generating a protein complex capable of supporting robust gene expression (Aoyagi and Archer, 2008; McKenna et al., 1998). Transcriptional coactivators harboring intrinsic enzymatic activity such as histone acetyltransferases (HATs), including CREB-binding protein/E1A-binding protein p300 (CBP/p300) and steroid receptor coactivator 1–3 (SRC1–3), contribute to the activation of transcription by acetylating histone tails to promote the formation of less compact chromatin (Spiegelman and Heinrich, 2004; Swygert and Peterson, 2014; Yaroslava and Bert, 2011). Additionally, HATs can also regulate gene transcription by directly acetylating transcription factors which modulates their transcriptional activity or DNA binding affinity (Fang et al., 2008; Jin et al., 2011; Karamouzis et al., 2007; Ogryzko et al., 1996;

Abbreviations: HBV, hepatitis b virus; PGC1, peroxisome proliferator-activated receptor gamma coactivator 1; HepG2, human hepatoma cells; HEK293T, human embryonic kidney cells; HATs, histone acetyltransferases; CBP, CREB-binding protein; p300, E1A-binding protein; SRC1–3, steroid receptor coactivator 1–3; PRMTs, protein arginine methyltransferases; PPRC1, peroxisome proliferator-activated receptor γ coactivator-related 1; PPARα, peroxisome proliferator-activated receptor α; ERRα, estrogen-related receptor α; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; Fox, forkhead box transcription factor; Sp1, specificity protein 1 transcription factor; NR, nuclear receptor transcription factor; HNF4, hepatocyte nuclear factor 4; RXR, retinoid X receptor; FXR, farnesoid X receptor; LRH1, liver receptor homolog 1; TBP, TATA-binding protein

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Spencer et al., 1997; Zhang and Bieker, 1998). Likewise, protein arginine methyltransferases (PRMTs) such as PRMT1 and PRMT4 regulate transcriptional activity of their target genes by catalyzing arginine methylation of histone tails, transcription factors and other coactivators (An et al., 2004; Barrero and Malik, 2006; Kleinschmidt et al., 2008; Teyssier et al., 2005).

Alternatively some coactivators, such as the PGC1 family members, lack any apparent enzymatic activity and hence function as structural scaffolds for the recruitment of other components of the transcriptional complex (Puigserver et al., 1999; Spiegelman and Heinrich, 2004; Yaroslava and Bert, 2011). The PGC1 family of coactivators comprises three members: PGC1α, PGC1β and peroxisome proliferator-activated receptor γ coactivator-related 1 (PPRC1). PGC1 coactivators utilize distinct functional protein domains to interact with gene promoter-bound nuclear receptors and recruit additional coactivators with various enzymatic activities to modulate gene expression (Heery et al., 1997; Knutti et al., 2000; Kressler et al., 2002; Puigserver et al., 1999; Teyssier et al., 2005; Villena, 2015). PGC1α and PGC1β share extensive sequence similarities especially towards their amino termini which harbor nuclear receptor binding motifs and coactivator interaction domains (Lin et al., 2005, 2002, 2003; Puigserver et al., 1999; Villena, 2015). Despite these similarities, PGC1α and PGC1β may differentially affect nuclear receptor-mediated gene expression due to their differential affinities for various nuclear receptors and susceptibility to modulation by distinct signal transduction pathways at both the transcriptional and posttranscriptional levels (Adamovich et al., 2013; Fujita et al., 2015; Gao et al., 2015; Lin et al., 2003; Villena, 2015).

Previously, it was shown that PGC1α enhances HBV transcription and replication in human hepatoma cell lines and differentially modulates nuclear receptor-mediated HBV biosynthesis in HEK293T cells (Ondracek and McLachlan, 2011; Ondracek et al., 2009). Additionally, PGC1α can independently activate HBV biosynthesis in HEK293T cells by serving as an adaptor molecule for the recruitment of additional coactivators to the viral nucleocapsid promoter (Shalaby et al., 2017). These findings demonstrated that PGC1α-mediated gene regulation could represent an important pathway governing HBV transcription and replication. However, the observation that some transcription factor targets of PGC1α, including peroxisome proliferator-activated receptor α (PPARα) and estrogen-related receptor α (ERRα) can also recruit PGC1β suggested PGC1β might also have a critical role in regulating HBV biosynthesis under certain circumstances (Kamei et al., 2003; Lin et al., 2003; Schreiber et al., 2004; Vega et al., 2000; Villena, 2015). In the current study, it is demonstrated that PGC1β can only very modestly activate HBV biosynthesis. However, PGC1β can inhibit PGC1α-mediated HBV biosynthesis. This suggests that the competitive binding of PGC1α and PGC1β to nuclear receptors (and possibly additional transcription factors) at the HBV nucleocapsid promoter may determine the levels of HBV transcription and replication under various physiologically relevant conditions.

2. Materials and methods

2.1. Plasmid constructions

The HBV DNA (4.1kbp) construct that contains 1.3 copies of the HBV genome includes the viral sequence from nucleotide coordinates 1072–3182 plus 1–1990 (Tang and McLachlan, 2001). The pcDNA-HA-hPGC1α, pcDNA-HA-hPGC1β, pSG5-HA-CBP, pIRES-FHneoP300, pSG5-HA-SRC1e, pSG5-HA-GRIP1(SRC2), pIRESneoPRMT1, and pIRESneo CARM1(PRMT4) vectors express PGC1α, PGC1β, CBP, P300, SRC1, SRC2, PRMT1 and PRMT4 from the corresponding cDNAs, respectively, using the CMV immediate-early promoter (pcDNA3 and pIRESneo) or the simian virus 40 early promoter (pSG5) (An et al., 2004; Huang and Cheng, 2004; Kalkhoven et al., 1998; Knutti et al., 2000; Li et al., 2004; Shi et al., 2016).

2.2. Cells and transfections

The human hepatoma HepG2 cell line and human embryonic kidney 293T cell line were grown in RPMI-1640 medium and 10% fetal bovine serum at 37 °C in 5% CO_2/air. Transfections for viral RNA and DNA analysis were performed as previously described (McLachlan et al., 1987) using 10 cm plates, containing approximately 1×10^6 cells. DNA and RNA isolation was performed 3 days post transfection. The transfected DNA mixture was composed of either 5 or 10 μg of HBV DNA (4.1kbp) for HepG2 or 293T cell lines, respectively, plus various amounts of either pcDNA-HA-hPGC1α or pcDNA-HA-hPGC1β transcriptional coactivator expression vectors or both as indicated. Additionally, 1 μg of each of the transcriptional coactivator expression vectors pSG5-HA-CBP, pIRES-FHneoP300, pSG5-HA-SRC1e, pSG5-HA-SRC2 pIRESneoPRMT1 and pIRESneoPRMT4 (An et al., 2004; Huang and Cheng, 2004; Kalkhoven et al., 1998; Knutti et al., 2000; Li et al., 2004; Shi et al., 2016; Tang and McLachlan, 2001) were utilized in the transfection assays, as indicated. Controls were derived from cells transfected with HBV DNA and the expression vectors lacking a transcriptional coactivator cDNA insert (Raney et al., 1997).

2.3. Characterization of HBV transcripts and replication intermediates

Transfected cells from a single plate were divided equally and used for the preparation of total cellular RNA and viral DNA replication intermediates as described previously (Summers et al., 1991) with minor modifications. RNA (Northern) and DNA (Southern) filter hybridization analysis were performed using 20 μg of total cellular RNA and 30 μl of viral DNA replication intermediates, respectively, as described (Sambrook et al., 1989). Filter hybridization analyses were quantitated by phosphorimaging using a Molecular Dynamics Storm 5000 Phosphor Imager system.

2.4. Analysis of PGC1 protein levels by Immunoblotting

HepG2 and 293T cell lines were transfected with 10 μg of either pcDNA-HA-hPGC1α or pcDNA-HA-hPGC1β expression vectors. Twentyfour hours post transfection, cells were treated with 5 μM MG132 (Sigma-Aldrich) (Trausch-Azar et al., 2010). An additional 24 h later, cells were lysed in 300 μl Laemmli (2×) buffer (65.8 mM Tris-HCL, pH 6.8, 26.3% (w/v) glycerol, 2.1% SDS, 10.0% (v/v) 2-mercaptoehtanol). Total cellular protein (25 μg) was resolved by 4–8% SDS-PAGE and transferred to Immobilon PVDF membrane (Millipore) (Mao et al., 2013). The membranes were probed with HA-Tag rabbit monoclonal antibody (Cell Signaling Technology #3724, 1:1000 dilution) and GAPDH rabbit monoclonal antibody (Cell Signaling Technology #5174, 1:2000 dilution) followed by incubation with horseradish peroxidase-labeled goat anti-rabbit IgG (Cell Signaling Technology #7074, 1:2000). HA-tagged and GAPDH polypeptides were detected using enhanced chemiluminescence (Thermo Fisher Scientific #34080) as described by the manufacturer and quantitated using the ChemiDoc MP Imaging System (BioRad).

2.5. Statistics

All the data are presented as mean ± standard deviations from two independent experiments except where noted in the figure legend. Comparisons were performed using two tailed Student's *t*-test using Microsoft Excel software.

3. Results

3.1. Differential modulation of HBV biosynthesis by PGC1α and PGC1β in human hepatoma HepG2 cells

The effects of PGC1α and PGC1β on HBV biosynthesis were initially

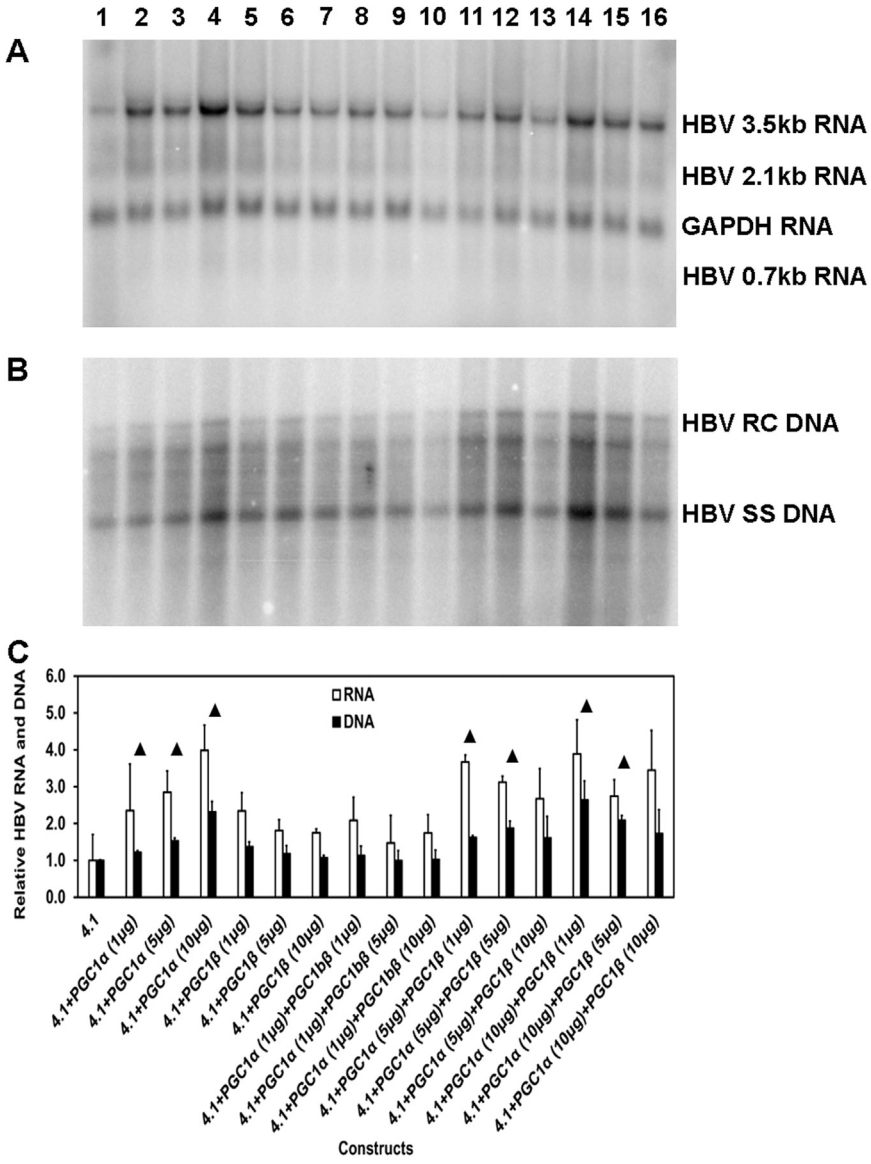

Fig. 1. Effects of PGC1α and PGC1β expression on HBV biosynthesis in the human hepatoma cell line, HepG2. (A) RNA (Northern) filter hybridization analysis of HBV transcripts. The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcript was used as an internal control for RNA loading per lane. (B) DNA (Southern) filter hybridization analysis of HBV replication intermediates. HBV RC DNA, HBV relaxed circular DNA; HBV SS DNA, HBV single-stranded DNA. Cells were transfected with the HBV DNA (4.1kbp) construct (lanes 1–16) plus PGC1α (lanes 2–4 and 8–16), PGC1β (lanes 5–16) expression vectors, as indicated. (C) Quantitative analysis of the HBV 3.5-kb RNA and HBV DNA replication intermediates. The levels of the HBV 3.5-kb RNA and total HBV DNA replication intermediates are reported relative to the value for the HBV DNA (4.1kbp) construct (lane 1). The mean RNA and DNA levels plus standard deviations from two independent analyses are shown (n = 2). Levels of the transcripts (lane 11) and replication intermediates (lanes 2–4, 11, 12, 14 and 15) in PGC1-expressing cells that are statistically significantly higher than the levels in cells transfected with the HBV DNA (4.1kbp) construct alone (lane 1), as determined by Student's *t*-test ($P < 0.05$), are indicated with a triangle (▲).

examined utilizing the human hepatoma cell line, HepG2. Transfection of the HBV DNA (4.1kbp) construct into HepG2 cells supports HBV transcription and replication (Fig. 1A and B, lane 1). Exogenous expression of increasing levels of PGC1α led to a dose-dependent enhancement of viral 3.5-kb RNA and DNA replication intermediates levels as reported previously (Ondracek and McLachlan, 2011) (Fig. 1A and B, lanes 2–4). In contrast, expression of increasing levels of PGC1β had a very limited effect on HBV transcription and replication (Fig. 1A and B, lanes 5–7) suggesting that PGC1α is a more potent activator of HBV transcription and replication than PGC1β in HepG2 cells. However expression of increasing levels of PGC1β in the presence of exogenously expressed PGC1α reduced PGC1α-dependent HBV transcription and replication to a modest extent (Fig. 1A and B, lanes 8–16). These observations suggest that PGC1β may negatively regulate PGC1α-mediated HBV biosynthesis in HepG2 cells. Due to high constitutive HBV biosynthesis and the modest PGC1β-mediated effects on HBV RNA and DNA synthesis in HepG2 cells, a nonhepatoma HBV replication system was utilized to examine further the relative contribution of PGC1β to HBV biosynthesis.

3.2. PGC1β is a poor transcriptional coactivator of HBV biosynthesis in human embryonic kidney HEK293T cells

In contrast to HepG2 cells (Fig. 1A and B, lane 1), transfection of the HBV DNA (4.1kbp) construct into HEK293T cells does not produce detectable viral replication intermediates (Fig. 2A and B, lane 1). However, cotransfection of the HBV DNA (4.1kbp) construct with the PGC1β expression vector led to an approximately two-fold increase in HBV 3.5-kb RNA levels and detectable HBV DNA synthesis (Fig. 2A and B, lane 2). In contrast, expression of the individual coactivators CBP, SRC1 or PRMT1 with the HBV DNA (4.1kbp) construct does not appear to modulate pregenomic RNA levels and fails to support readily detectable viral replication intermediates (Fig. 2A and B, lanes 3–5). Various combinations of the coactivators CBP, SRC1 and PRMT1 generally supported a limited increase in PGC1β-mediated HBV biosynthesis (Fig. 2A and B, lanes 6–8, 12–14 and 16). The only additional combination of coactivators capable of supporting viral biosynthesis included CBP plus SRC1 (Fig. 2A and B, lanes 9 and 15). Overall, these observations suggest that PGC1β may function as an adaptor molecule for the recruitment of additional endogenous or exogenously expressed coactivators required for HBV pregenomic 3.5-kb RNA synthesis and viral replication. However the effects of PGC1β on viral biosynthesis are

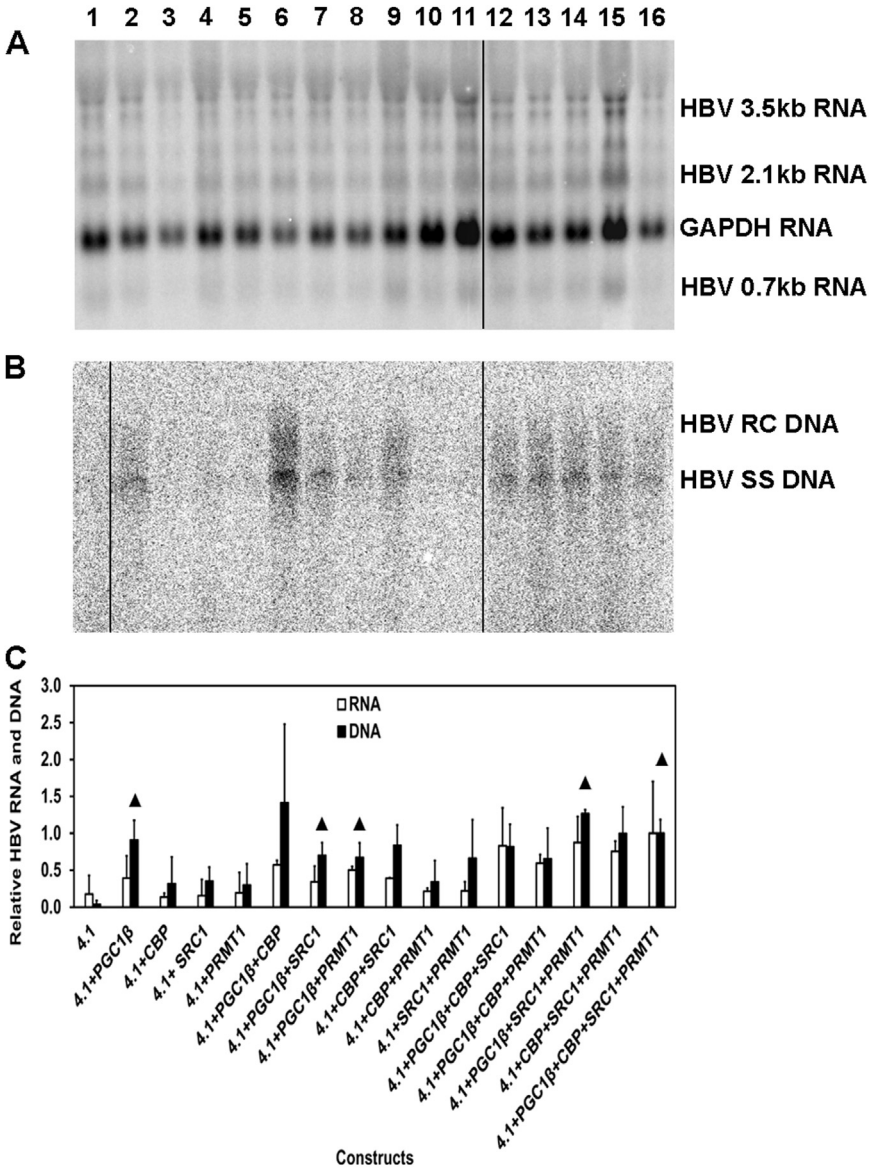

Fig. 2. Effect of PGC1β expression on HBV biosynthesis in the presence and absence of additionally expressed transcriptional coactivators in the human embryonic kidney cell line, HEK293T. (A) RNA (Northern) filter hybridization analysis of HBV transcripts. The 3.9-kb transcript observed above the HBV 3.5-kb RNA probably represents the previously reported HBV long xRNA that initiates from the X promoter region (Doitsh and Shaul, 2003). The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcript was used as an internal control for RNA loading per lane. The black line indicates noncontiguous lanes from a single filter hybridization analysis. (B) DNA (Southern) filter hybridization analysis of HBV replication intermediates. HBV RC DNA, HBV relaxed circular DNA; HBV SS DNA, HBV single-stranded DNA. Cells were transfected with the HBV DNA (4.1kbp) construct (lanes 1–16) plus PGC1β (lanes 2, 6–8, 12–14 and 16), CBP (lanes 3, 6, 9, 10, 12, 13, 15, and 16), SRC1 (lanes 4, 7, 9, 11, 12, and 14–16), and PRMT1 (lanes 5, 8, 10, 11, and 13–16) expression vectors, as indicated. The black line indicates noncontiguous lanes from a single filter hybridization analysis. (C) Quantitative analysis of the HBV 3.5-kb RNA and HBV DNA replication intermediates. The levels of the HBV 3.5-kb RNA and total HBV DNA replication intermediates are reported relative to the value for the HBV DNA (4.1kbp) construct in the presence of the expression of four coactivators (lane 16). The mean RNA and DNA levels plus standard deviations from two independent analyses are shown (n = 2). Levels of replication intermediates (lanes 2, 7, 8, 14 and 16) in coactivator-expressing cells that are statistically significantly higher than the levels in cells transfected with the HBV DNA (4.1kbp) construct only (lane 1), as determined by Student's *t*-test ($P < 0.05$), are indicated with a triangle (▲).

relatively modest compared with the effects elicited by PGC1α expression in HEK293T cells (Figs. 3 and 4).

3.3. Effects of various transcriptional coactivator combinations on PGC1α- and PGC1β-mediated HBV biosynthesis in human embryonic kidney HEK293T cells

Members of the CBP, SRC and PRMT classes of coactivators have been shown to potentiate PGC1α-mediated cellular transcriptional programs (Puigserver et al., 1999; Teyssier et al., 2005; Wallberg et al., 2003). Additionally, it has been demonstrated previously that the transcriptional coactivators CBP, SRC1 and PRMT1 can enhance PGC1α-mediated HBV biosynthesis (Shalaby et al., 2017). However, their effects on PGC1β-mediated HBV biosynthesis are quite limited (Fig. 2). Consequently, it was of interest to compare the effects of alternative coactivator combinations on PGC1α- and PGC1β-mediated HBV biosynthesis. Irrespective of the combination of coactivators being used, PGC1α expression produced greater levels of HBV 3.5-kb RNA and DNA replication intermediates than the levels produced by PGC1β expression (Fig. 3). These data indicate that PGC1α is a more robust coactivator of HBV biosynthesis than PGC1β in HEK293T cells. This difference might be attributed, in part, to the ability of PGC1α to recruit coactivators and general transcription factors more efficiently than PGC1β to the HBV nucleocapsid promoter, supporting greater pregenomic RNA synthesis.

3.4. PGC1β inhibits PGC1α-mediated HBV biosynthesis in human embryonic kidney HEK293T cells

In HepG2 cells, expression of increasing levels of PGC1β in the presence of exogenously expressed PGC1α slightly decreased PGC1α-mediated HBV transcription and replication (Fig. 1). Based on this observation, it was of interest to investigate the effect of potential cross-talk between PGC1α and PGC1β on HBV biosynthesis in non-hepatoma HEK293T cells. Transfection of the HBV DNA (4.1kbp) construct into HEK293T cells supports limited expression of HBV pregenomic 3.5-kb RNA but viral replication intermediates are undetectable (Fig. 4A and B, lane 1) (Shalaby et al., 2017). Expression of PGC1α led to an approximately two-fold increase in HBV 3.5-kb RNA and robust activation of viral replication (Fig. 4A and B, lane 2) (Shalaby et al., 2017). However, the effects of PGC1β expression on HBV biosynthesis were very limited by comparison with PGC1α (Fig. 4A and B, lanes 3–5). Importantly, expression of increasing levels of PGC1β inhibited PGC1α-mediated HBV 3.5-kb RNA accumulation and viral replication

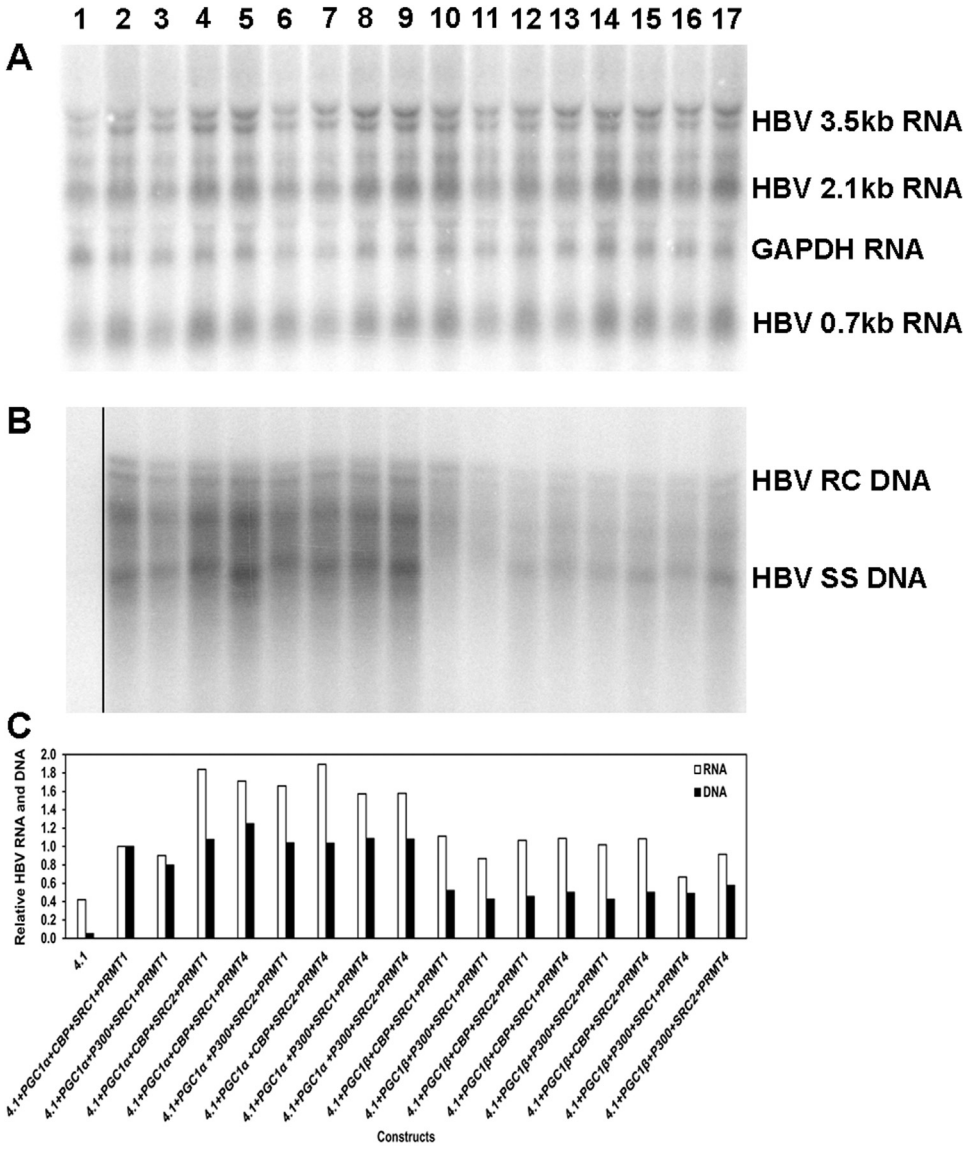

Fig. 3. Effects of PGC1α versus PGC1β expression on HBV biosynthesis in the presence of various transcriptional coactivator combinations in the human embryonic kidney cell line, HEK293T. (A) RNA (Northern) filter hybridization analysis of HBV transcripts. The 3.9-kb transcript observed above the HBV 3.5-kb RNA probably represents the previously reported HBV long xRNA that initiates from the X promoter region (Doitsh and Shaul, 2003). The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcript was used as an internal control for RNA loading per lane. (B) DNA (Southern) filter hybridization analysis of HBV replication intermediates. HBV RC DNA, HBV relaxed circular DNA; HBV SS DNA, HBV single-stranded DNA. Cells were transfected with the HBV DNA (4.1kbp) construct (lanes 1–17) plus PGC1α (lanes 2–9), PGC1β (lanes 10–17), CBP (lanes 2, 4, 5, 7, 10, 12, 13 and 15), SRC1 (lanes 2, 3, 5, 8, 10, 11, 13 and 16), PRMT1 (lanes 2–4, 6, 10–12 and 14), P300 (lanes 3, 6, 8, 9, 11, 14, 16 and 17), SRC2 (lanes 4, 6, 7, 9, 12, 14, 15 and 17) and PRMT4 (lanes 5, 7–9, 13 and 15–17) expression vectors, as indicated. The black line indicates noncontiguous lanes from a single filter hybridization analysis. (C) Quantitative analysis of the HBV 3.5-kbp RNA and HBV DNA replication intermediates. The levels of the HBV 3.5-kbp RNA and total HBV DNA replication intermediates are reported relative to the value for the HBV DNA (4.1kbp) construct in the presence of the expression of PGC1α, CBP, SRC1 and PRMT1 (lane 2). Levels of transcripts and replication intermediates in PGC1α-expressing cells are statistically significantly higher than the levels in PGC1β expressing-cells as determined by Student's *t*-test ($n = 8$; $P < 0.01$).

intermediate levels by up to 10-fold (Fig. 4A and B, lanes 6–8). These data support the suggestion that PGC1β is a poor activator of HBV transcription and replication but an efficient antagonist of PGC1α-mediated HBV biosynthesis (Figs. 1–4).

Based on the different effects of PGC1α and PGC1β on HBV transcription and replication (Figs. 1–4), it was important to determine if their expression levels influenced their activities in HepG2 and HEK293T cells. MG132 treatment was used to inhibit proteasome-mediated degradation of PGC1 polypeptides (Fig. 5) (Fujita et al., 2015; Trausch-Azar et al., 2010). This treatment was necessary to detect PGC1 polypeptide expression in HepG2 cells (Fig. 5A). As demonstrated previously (Puigserver et al., 1998; Sano et al., 2007), the higher molecular weight bands detected at approximately 125 and 180 kDa correspond to the full-length PGC1α and PGC1β polypeptides, respectively (Fig. 5A and B). The PGC1α and PGC1β related polypeptides detected at approximately 75 and 95 kDa probably represent degradation products derived from full length PGC1α and PGC1β, respectively (Fig. 5A and B). PGC1α and PGC1β were only detected in HepG2 cells in the presence of the proteasome inhibitor, MG132 (Fig. 5A). In contrast, PGC1α and PGC1β were detected in HEK293T cells in the presence or absence of the proteasome inhibitor, MG132, although higher levels of PGC1 polypeptides were detected with MG132 treatment (Fig. 5B). Collectively, these data are consistent with previous reports indicating that PGC1α and PGC1β are subject to ubiquitination-mediated proteasome proteolysis (Fujita et al., 2015; Sano et al., 2007; Trausch-Azar et al., 2010). In general, PGC1β was expressed at approximately 3-fold levels higher than PGC1α under similar conditions (Fig. 5). Based on these limited differences in PGC1α and PGC1β polypeptide expression, it appears that these two related coactivators have distinct functional effects on HBV biosynthesis. Accordingly, PGC1α is a more potent activator of HBV biosynthesis than PGC1β but PGC1β can inhibit viral transcription and replication by competing with PGC1α during the assembly of the transcriptional pre-initiation complex at the HBV nucleocapsid promoter.

4. Discussion

Transcriptional coactivators play an important role in promoting stable preinitiation complex assembly on gene promoter sequences and hence, modulate transcriptional outputs (Aoyagi and Archer, 2008). However, the effects of recruiting distinct coactivator complexes by promoter-bound transcription factors on HBV biosynthesis have not been examined in detail (Shalaby et al., 2017). Investigating the effects of distinct coactivators and their functional crosstalk on HBV biosynthesis could guide the identification of novel transcriptional targets for the development of antiviral therapeutic compounds aimed at

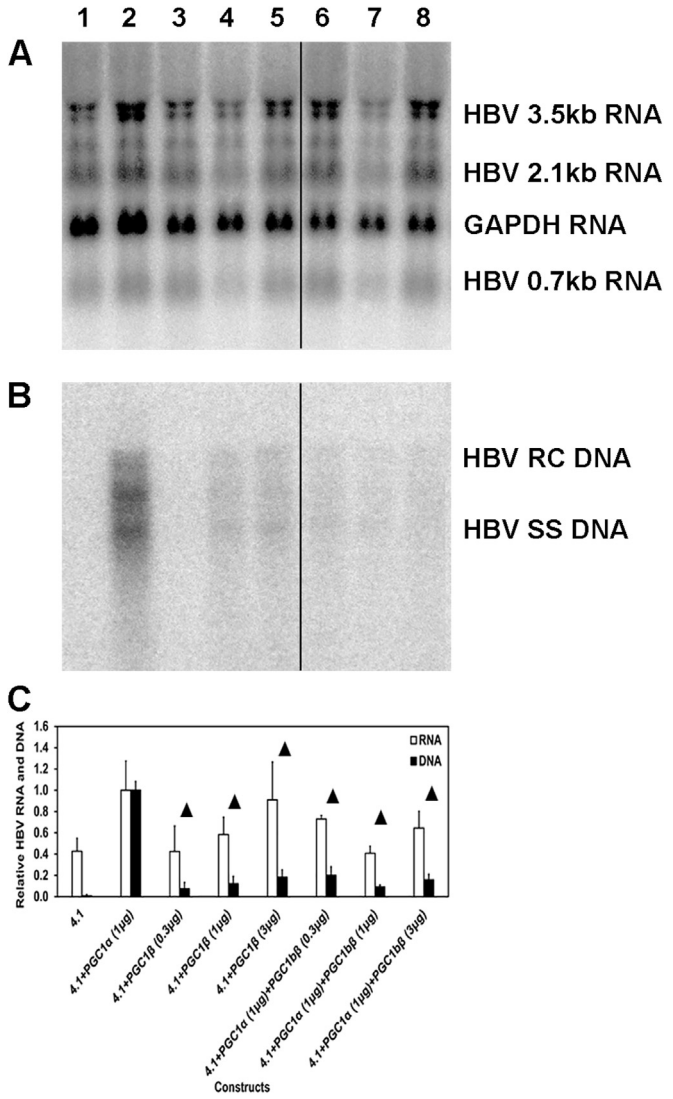

Fig. 4. PGC1β inhibition of PGC1α-dependent HBV biosynthesis in the human embryonic kidney cell line, HEK293T. (A) RNA (Northern) filter hybridization analysis of HBV transcripts. The 3.9-kb transcript observed above the HBV 3.5-kb RNA probably represents the previously reported HBV long xRNA that initiates from the X promoter region (Doitsh and Shaul, 2003). The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcript was used as an internal control for RNA loading per lane. The black line indicates noncontiguous lanes from a single filter hybridization analysis. (B) DNA (Southern) filter hybridization analysis of HBV replication intermediates. HBV RC DNA, HBV relaxed circular DNA; HBV SS DNA, HBV single-stranded DNA. Cells were transfected with the HBV DNA (4.1kbp) construct (lanes 1–8) plus PGC1α (lanes 2 and 6–8), PGC1β (lanes 3–8) expression vectors, as indicated. The black line indicates noncontiguous lanes from a single filter hybridization analysis. (C) Quantitative analysis of the HBV 3.5-kb RNA and HBV DNA replication intermediates. The levels of the 3.5-kb HBV RNA and total HBV DNA replication intermediates are reported relative to the value for the HBV DNA (4.1kbp) construct in the presence of PGC1α expression (lane 2). The mean RNA and DNA levels plus standard deviations from two independent analyses are shown. Levels of the replication intermediates (lanes 3–8) in PGC1β-expressing cells that are statistically significantly lower than the levels in cells transfected with the HBV DNA (4.1kbp) construct plus 1 μg of PGC1α expression vector (lane 2), as determined by Student's *t*-test ($P < 0.05$), are indicated with a triangle (▲).

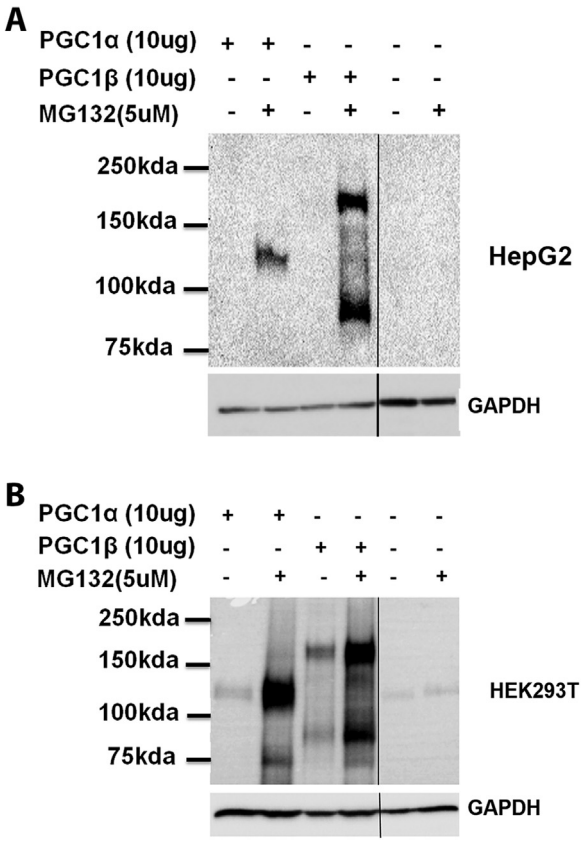

Fig. 5. Proteasome inhibitors stabilize PGC1α and PGC1β proteins in the human hepatoma cell line, HepG2 and the human embryonic kidney cell line, HEK293T. (A) HepG2 cells and (B) HEK293T cells were transfected with the expression vectors HA-PGC1α or HA-PGC1β. Transfected cells were treated or untreated with MG132 (5 μM) for 24hrs. Total cell lysates were subjected to immunoblotting using anti-HA antibodies. Controls were derived from untransfected cells. The black line indicates noncontiguous lanes from a single filter immuno-detection analysis.

resolving chronic HBV infection. The transcriptional coactivator, PGC1α has been shown to activate HBV transcription and replication both in vivo and in mammalian cell lines (Amir et al., 2006; Ondracek et al., 2009; Shalaby et al., 2017). In contrast, it is unclear to what extent its structural homolog, PGC1β, can regulate HBV biosynthesis.

Here it is demonstrated that PGC1α and PGC1β can differentially regulate HBV biosynthesis (Fig. 6). In both HepG2 and HEK293T cells, PGC1α is a more potent activator of HBV biosynthesis than PGC1β. Binding of PGC1α to the transcription factors, including endogenous nuclear receptors and forkhead box transcription factor family members, associated with the HBV nucleocapsid promoter produces higher levels of HBV biosynthesis than occurs with PGC1β (Fig. 6). Based upon previous and current analyses (Shalaby et al., 2017), PGC1-mediated HBV transcription and replication is dependent upon the ability of PGC1 coactivators to serve as adaptor molecules for the recruitment of additional coactivators to stimulate viral RNA synthesis. PGC1 coactivators can recruit endogenous or exogenously expressed coactivators to stimulate HBV biosynthesis (Fig. 6). Co-expression of histone acetyltransferses including CBP/p300 and SRC1 and 2 plus protein arginine methyltransferases, PRMT1 and 4, in all possible combinations enhanced PGC1-dependent HBV biosynthesis. However, PGC1α-dependent HBV biosynthesis was always greater than PGC1β-dependent HBV biosynthesis (Fig. 3).

Importantly, PGC1β was capable of inhibiting PGC1α-mediated HBV biosynthesis in a dose dependent manner when both coactivators were co-expressed (Fig. 6). This novel regulatory relationship between PGC1α and PGC1β appears to reflect a direct competition of these two related coactivators for common endogenous nuclear receptors or other transcription factors involved in controlling initiation of RNA synthesis from the HBV nucleocapsid promoter (Fig. 6). The net effect of this

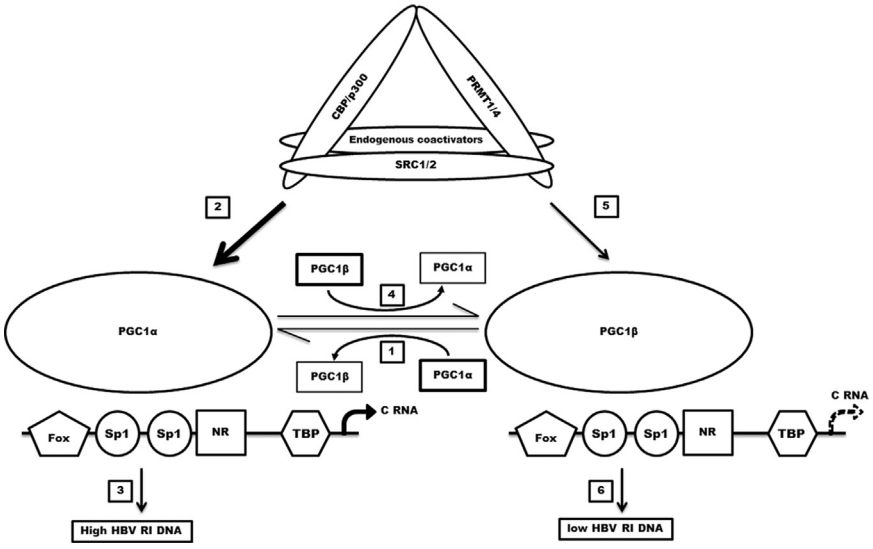

Fig. 6. Diagrammatic representation of the differential effects of PGC1 coactivators on HBV nucleocapsid promoter activity and viral replication intermediate (RI) DNA. C RNA, HBV pregenomic or core 3.5-kb RNA; Fox, forkhead box transcription factor (Johnson et al., 1995); Sp1, specificity protein 1 transcription factor (Zhang et al., 1993); NR, nuclear receptor transcription factor (i.e., HNF4, RXR, PPAR, FXR, LRH1, and estrogen-related receptor (ERR)) (Raney et al., 1997; Reese et al., 2011a; Tang and McLachlan, 2001); TBP, TATA-binding protein. High PGC1α activity relative to PGC1β [1] results in efficient recruitment of additional coactivators including exogenously expressed CBP/p300, SRC1/2 and PRMT1/4 plus endogenous coactivators to the HBV nucleocapsid promoter [2] leading to robust HBV RNA and DNA synthesis [3]. In contrast, high PGC1β activity relative to PGC1α [4] results in inefficient recruitment (or activation) of additional coactivators including exogenously expressed CBP/p300, SRC1/2 and PRMT1/4 plus endogenous coactivators to the HBV nucleocapsid promoter [5] leading to limited HBV RNA and DNA synthesis [6]. Competition between PGC1α and PGC1β determines the level of HBV biosynthesis.

competition on HBV biosynthesis depends upon the effective concentrations of PGC1α and PGC1β and their relative affinities for the transcription factors with which they associate. In addition PGC1α may either more efficiently recruit the same coactivators as PGC1β to the HBV nucleocapsid promoter or it may recruit a different set of coactivators than PGC1β, resulting in more robust transcription from the HBV nucleocapsid promoter. In support of the former suggestion, PGC1α and PGC1β displayed differential binding affinities and coactivation activities when regulating gluconeogenic gene expression through their interaction with the nuclear receptor, HNF4α (Lin et al., 2003). Additional analysis will be required to determine the mechanism of differential activation of HBV biosynthesis by the PGC1 coactivators. Regardless of the reasons for the difference in the levels of PGC1-dependent HBV biosynthesis, these findings imply that physiological stimuli that modulate the relative activities of PGC1α and PGC1β are likely to result in differences in viral biosynthesis.

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