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Transcriptional Coactivators CBP and p300 Cooperatively Enhance HNF-1α–Mediated Expression of the Albumin Gene in Hepatocytes

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Transcriptional coactivators, CREB-binding protein (CBP) and p300, exhibit high homology in structure and similar functions. In the present study, we analyzed the function of CBP and p300 proteins as transcriptional coactivators in the expression of albumin in hepatocytes. The expression levels of CBP and p300 were high in fetal hepatocytes, but low in adult ones. Immunoprecipitation assays showed that both CBP and p300 interacted with hepatocyte nuclear factor-1α (HNF-1α) in primary hepatocytes. Furthermore, CBP and p300 were co-precipitated without HNF-1α. Chromatin immunoprecipitation (ChIP) assays revealed that both CBP and p300 are located in the albumin promoter region in hepatocytes. These results suggested that HNF-1α, CBP and p300 were incorporated into a preinitiation complex of RNA polymerase II at the albumin promoter. Luciferase reporter assays showed that CBP and p300 cooperatively triggered HNF-1α–mediated transcription of the albumin promoter. In addition, inhibition of CBP or p300 using small interfering RNAs (siRNAs) resulted in a reduction in albumin expression. These results suggest that both CBP and p300 are required for enhanced expression of albumin.

Key words: CBP, HNF-1α, primary hepatocytes, p300.

The coactivators CREB-binding protein (CBP) and p300, which were originally identified as partners for cAMP response element binding protein (CREB) (*1*) and adenoviral E1A protein (*2*), respectively, possess intrinsic histone acetyltransferase (HAT) activity. In addition, they recruit other cofactors possessing HAT activity via protein-protein interactions, such as p300/CBP associated factor (P/CAF), ACTR, SRC-1 and p/CIP (*3*, *4*). The importance of CBP and p300 in transcriptional regulation is also supported by their ability to interact with a large number of transcription factors, including nuclear hormone receptors (*5*), AP1 (*6*), Myb (*7*), Jun (*8*), Fos (*9*), MyoD (*10*, *11*), p53 (*12*, *13*), CCAAT/enhancer-binding protein β (C/EBPβ) (*14*, *15*), and others. It is generally believed that the interaction of these factors with CBP and p300 provides a mechanism that localizes the coactivators to specific DNA regions, resulting in site-specific histone acetylation, chromatin remodeling and the activation of specific genes. Furthermore, CBP and p300 have been found to interact with TFIIB, TBP and RNA polymerase II. These coactivator proteins seem to function as components of the transcriptional initiation complex by bridging between transcriptional activators and the basal transcription apparatus (*16*, *17*). Thus, recruitment of CBP/p300 by DNA-bound transcription factors could facilitate the formation of a preinitiation complex at relevant promoters.

Hepatocyte nuclear factor-1 (HNF-1) is a transcriptional regulator composed of HNF-1α/HNF-1β hetero- or homo-dimers. These homeoproteins share an identical DNA-binding domain, but possess different transcriptional activation properties (*18*). HNF-1α is one of the most important transactivators in liver-specific gene expression including albumin transcription (*19*, *20*). Interestingly, HNF-1α, but not HNF-1β, is expressed in hepatocytes. Thus, the HNF-1α homo-dimer operates in the liver, whereas hetero-dimers are detected in other organs (*21*, *22*). Using a luciferase reporter assay system with a synthetic albumin promoter, Soutoglou *et al.* found that HNF-1α recruited CBP and P/CAF, and that the HAT activity of the proteins was required for effective transactivation (*23*, *24*).

CBP and p300 had been thought to have similar functions but the functional difference between them was unclear. In the present study, we examined the effects of CBP and p300 on liver-specific HNF-1α–mediated expression of the albumin gene in a physiological system involving primary hepatocytes and a model system involving cell lines. The results suggest that both these coactivator proteins are included in a protein complex in the albumin promoter region and act cooperatively to enhance the expression of the albumin gene.

MATERIALS AND METHODS

Isolation and Culture of Rat Hepatocytes—Adult rat hepatocytes were obtained from male Sprague-Dawley rats (6–7 weeks old; Japan SLC, Shizuoka) by the collagenase perfusion method (*25*, *26*). Williams' medium E (Invitrogen, San Diego, CA, USA) supplemented with 0.1 μM $CuSO_4 \cdot 5H_2O$, 25 nM Na_2SeO_3, 1.0 μM $ZnSO_4 \cdot 7H_2O$, 0.1 μM insulin (Sigma-Aldrich, St. Louis, MO, USA), 1.0

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μM dexamethasone (Wako Pure Chemical Industries, Osaka), 20 ng/ml of epidermal growth factor (EGF) (Sigma), 48 μg/ml of gentamicin sulfate (Sigma), and 100 μg/ml of chloramphenicol (Wako) was used as the culture medium. Fetal hepatocytes were isolated from the fetuses (17 embryonic days; E17) of Sprague-Dawley rats (Japan SLC) (*27*, *28*), and cultured in Williams' medium E containing 5% (v/v) fetal bovine serum. Cells were seeded onto collagen type I-coated plastic dishes (Iwaki Glass Works, Chiba) under a 5% CO_2 atmosphere.

SDS-PAGE and Western Blot Analysis—Total cellular proteins were extracted by adding extraction buffer (10 mM Tris-HCl, pH 8.0, containing 8 M urea and 0.1 M NaH_2PO_4) to the cell pellet obtained on centrifugation. After treatment of the sample solutions in SDS-PAGE sample buffer, samples (100 μg of total cell protein) were electrophoresed on a 6% (for CBP and p300) or 10% (for HNF-1α) SDS-polyacrylamide gel (*29*), and then transferred to a Hybond-PVDF membrane (Amersham Biosciences, Piscataway, NJ, USA). The membranes were incubated in Tris-buffered saline (TBS) (10 mM Tris-HCl, pH 8.0, containing 150 mM NaCl) containing 5% skim milk and 0.05% Tween 20 to block the remaining protein binding sites. After a wash with TBS containing 0.05% Tween 20, the proteins were detected with anti-HNF-1α, anti-CBP or anti-p300 antibodies (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The specific antibodies were then detected with peroxidase-conjugated secondary antibodies (Santa Cruz Biotechnology) using an ECL detection kit (Amersham Biosciences).

Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Analysis—Total RNA was prepared from rat hepatocytes using a total RNA extraction kit (Amersham Biosciences), and then reverse transcribed with a reverse transcriptase (ReverTra Ace®; Toyobo, Osaka, Japan) to generate cDNAs. The detection of CBP and p300 cDNAs was performed by PCR using a pair of primers (5′-AGACTCATCAACACCCAGCC-3′ and 5′-GGTTGTCCAAACGGACTTGT-3′ for CBP; 5′-CCAACAGAATAGCCCTGGAT-3′ and 5′-CTGAATTTGGAGACCAAGGC-3′ for p300).

Plasmids—To construct an expression vector plasmid for C/EBPα, the rat C/EBPα gene was amplified from pMSV-C/EBPα (*30*) and introduced into pcDNA4/TO/myc-His (Invitrogen) (designated as pcDNA4-C/EBPα). For the C/EBPβ expression vector plasmid, the rat C/EBPβ gene was amplified from pSCT-LAP (*31*) and introduced into pcDNA4/TO/myc-His (designated as pcDNA4-C/EBPβ). For the HNF-1α expression vector plasmid, the rat HNF-1α gene was amplified from pHF22.1-HNF-1α (*32*) and introduced into pcDNA4/TO/myc-His (designated as pcDNA4-HNF-1α). pRc/RSV-CBP (*33*) and pCMV-p300 (UpstateBiotechnology, Lake Placid, NY, USA) (*2*) were used for the expression of CBP and p300, respectively. To construct an albumin promoter-luciferase reporter plasmid, 216 bp of the albumin promoter region was amplified from rat genomic DNA by PCR using a pair of primers (5′-GGTAAGCTTATTGACAAAGCCTTTGGG-3′ and 5′-CCTAAGCTTTTGACAGGAAAGGTGGTCTGTGT-3′) (*Hin*d III sites are underlined), and introduced into the pGL3-basic vector (Promega, Madison, WI, USA). The reporter plasmid was designated as pGL3-Palb.

Immunoprecipitation Assay—Primary hepatocytes or transfected C33A cells (5×10^6) were used for the immunoprecipitation reaction. C33A cells were co-transfected with 3.0 μg of expression vectors for HNF-1α and CBP or p300 by the calcium-phosphate method (*34*). The transfected C33A cells or hepatocytes were lysed in lysis buffer (50 mM Tris-HCl, pH7.5, containing 200 mM KCl, 30 mM $MgCl_2$, 10 mM EDTA and 0.5% NP-40) containing protease inhibitors (1 ng/ml of leupeptin, 1 ng/ml of aprotinin, 1 μg/ml of pepstatin A and 1 mM PMSF). The cell lysates were incubated on ice for 30 min and DNA was disrupted by sonication, followed by centrifugation at 12,000 × *g* for 30 min at 4°C. Then, the supernatants were incubated with 10 μl of protein G-Sepharose beads (Sigma) at 4°C overnight with rotation to remove protein G-binding protein in the samples. After removing the resin, 1 μg of the respective antibodies was added to the supernatants, followed by incubation at 4°C for 2 h with rotation. The immune complexes were adsorbed to 10 μl of protein G-Sepharose at 4°C for 3 h and then the beads were washed 5 times with the lysis buffer. To the gel beads, a half volume of SDS sample buffer was added. The solution was heated at 100°C for 5 min and then directly subjected to SDS-PAGE.

Luciferase Reporter Gene Assay—Human adrenal carcinoma SW13 cells seeded onto 24-well plates were transfected with 0.05 μg of the luciferase reporter plasmid and *Renilla* luciferase expression plasmid containing the promoter region of the elongation factor Iα gene (Promega) with or without 0.1 μg of the HNF-1α, CBP or p300 expression plasmid using lipofection reagent (Lipofectamine™ 2000; Invitrogen). The amount of DNA transfected into the cells was kept constant by adding the empty vector. Luciferase activity was measured with a dual luciferase assay kit (Promega) according to the manufacturer's instructions using a luminescencer-JNR II (Atto, Tokyo). The data were normalized using *Renilla* luciferase activity levels.

Chromatin Immunoprecipitation (ChIP) Assay—The ChIP assay was performed using protein A agarose (Upstate cell signaling solutions, Charlottesville, VA, USA) according to the procedure described by Shang *et al.* (*35*) with slight modification. Fetal and adult hepatocytes were treated with 1% formaldehyde at 37°C for 10 min. The crosslinking reaction was stopped by adding 0.125 M glycine. After being washed with cold phosphate-buffered saline (PBS) containing protease inhibitors, the cells were harvested and suspended in lysis buffer (50 mM Tris-HCl, pH 8.1, containing 1% SDS and 10 mM EDTA). The lysate was sonicated to generate DNA fragments with an average length of 200 to 1,000 bp. After removal of cell debris by centrifugation, the supernatant was precleared with protein A agarose beads in the presence of 1 mg/ml of BSA and 2 μg/ml of sonicated λDNA to reduce the non-specific background. After removal of the beads by centrifugation, 2 μg of antibodies was added to 1 ml of supernatant. The immune complexes were collected by adding protein A agarose beads and washed five times with the washing buffer. After extensive washing, the adsorbed complexes were recovered by treatment with elution buffer (1% SDS and 0.1 M $NaHCO_3$). Crosslinks were then broken in 200 mM NaCl by incubation at 65°C for 5 h. The resulting samples were treated with 40 μg/ml

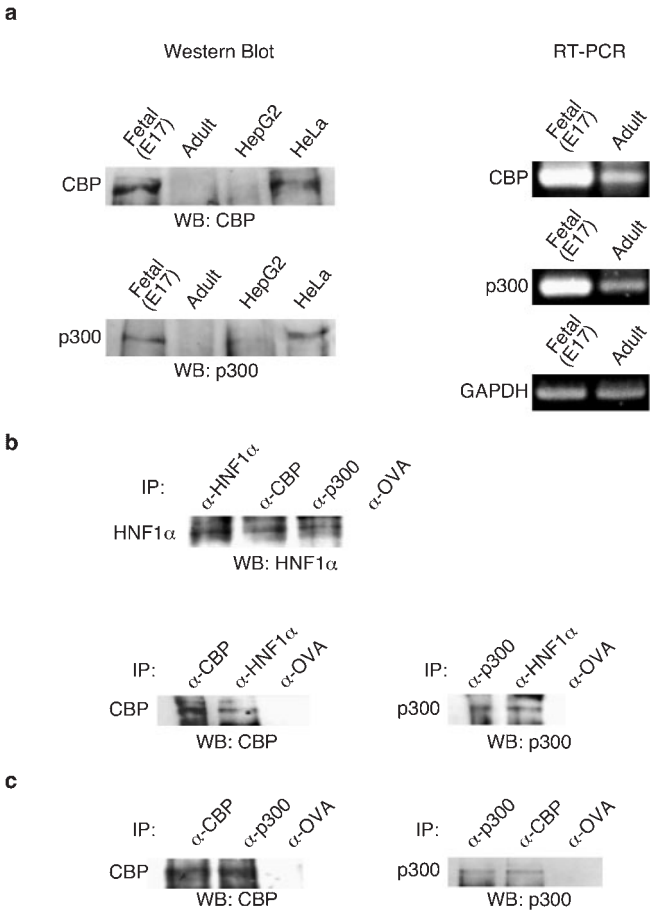

Fig. 1. **CBP and p300 interact with HNF-1α and with each other in primary hepatocytes.** (a) One hundred micrograms of protein from fetal and adult rat hepatocytes, HepG2 cells and HeLa cells, respectively, was electrophoresed on SDS-polyacrylamide gels. Western blot analysis was performed with anti-CBP and p300 antibodies. Detection of the RNAs of CBP and p300 was performed by RT-PCR. Total RNA was isolated from fetal (E17) and adult rat hepatocytes. (b) Co-immunoprecipitation of endogenous HNF-1α and CBP or p300. Extracts of fetal hepatocytes were immunoprecipitated with anti-HNF-1α, CBP or p300 antibodies. The right lane of each panel (anti-OVA antibody) shows immunoprecipitation with anti-OVA as a negative control. Western blot analysis was performed with anti-HNF-1α, CBP and p300 antibodies after SDS-PAGE. (c) Co-immunoprecipitation of CBP and p300 from fetal hepatocytes. Immunoprecipitates were detected with anti-CBP or anti-p300.

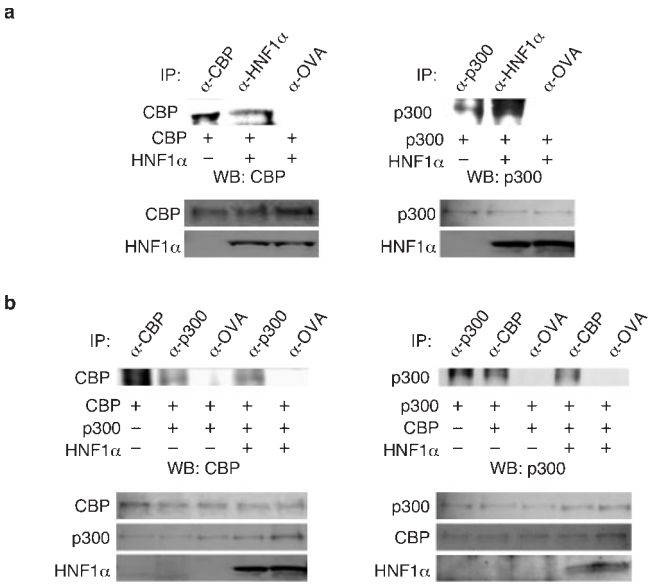

Fig. 2. **Interaction between CBP and p300 in transfected C33A cells.** C33A cells were transfected with 3 μg each of the HNF-1α, CBP and p300 expression vectors. Co-immunoprecipitation of HNF-1α and coactivator proteins (a), and CBP and p300 (b). The lower half is a Western blot of input proteins electrophoresed on a 6% (for CBP and p300) or 10% (for HNF-1α) SDS-polyacrylamide gel.

of proteinase K at 45°C for 30 min, followed by phenol/chloroform extraction and ethanol precipitation. The immunoprecipitated DNAs and input DNA were analyzed by PCR using primers (5′-CAAATGTGCCCTGCTTCCCTGC-3′ and 5′-GTGGGTTGACAGGAAAGGTGGTC-3′) for the albumin promoter. For PCR detection, after 4 min at 95°C, 34 cycles (fetal hepatocytes) or 37 cycles (adult hepatocytes) of 15 s at 96°C, 30 s at 60°C, and 30 s at 68°C were performed. Negative control experiments involving rabbit anti-OVA antibodies (Chemicon international, Temecula, CA, USA) for immunoprecipitation or involving primers for an albumin coding region (5′-TGTGTTTTCAAGGCTACCCTGA-3′ and 5′-GATGAAGGGAGGAAACCGAG-3′; 37 cycles) were performed, and the specific amplification was confirmed for each experiment.

Transfection of siRNAs—SiRNAs with the following sense and antisense sequences were used: HNF-1α (sense), 5′-AGUCUCGAUGACACCGUGG dTdT-3′; HNF-1α (antisense), 5′-CCACGGUGUCAUCGAGACUdTdT-3′, CBP (sense), 5′-CCCACAGCUAAUGGCAGCUdTdT-3′; CBP (antisense), 5′-AGCUGCCAUUAGCUGUGGGdTdT-3′, p300 (sense), 5′-CCCCAUGGAACAGCAUdTdT-3′; p300 (antisense), 5′-AUGCCCUUGGUUUUCCAUGGGGdTdT-3′. The synthetic siRNAs were purchased from Dharmacon Research (Lafayette, CO, USA), and introduced into cultured primary hepatocytes (30% confluent) using Oligofectoamine™ reagent (Invitrogen). SiRNA (100–200 pmol) was added to each well of a 24-well plate. Culture supernatants and cells were harvested after incubation for 48–72 h, and then the protein expression levels were determined by Western blotting.

Albumin Assay—The albumin concentration in the medium was determined with a sandwich solid-phase enzyme-linked immunosorbent assay (ELISA) using anti–rat albumin antibodies (Organon Teknika, Durham, NC, USA) and peroxidase-conjugated anti–rat albumin antibodies (Organon Teknika) as described previously (*25*, *28*). Purified rat albumin (Sigma) was used as a standard.

RESULTS

CBP and p300 Interact with Each Other in Primary Hepatocytes—The expression of the albumin gene is known to be controlled by HNF-1α and transcriptional coactivators such as CBP and P/CAF, as found in reporter assays involving NIH 3T3 and HepG2 cells (*23*, *24*). To

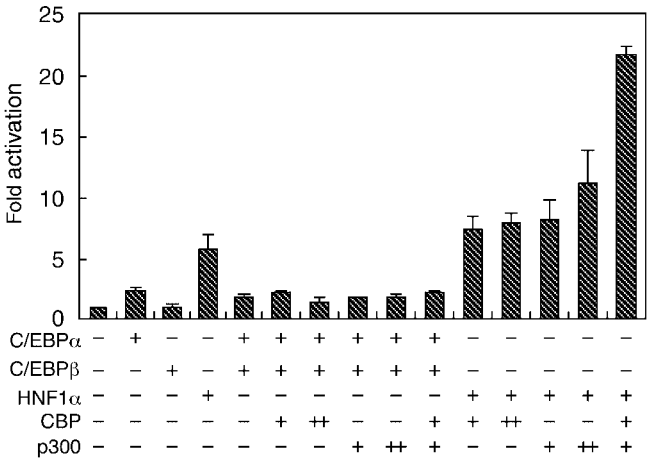

Fig. 3. **Both CBP and p300 are required for enhanced activation of HNF-1α–dependent transcription from the albumin promoter.** SW13 cells were co-transfected with 0.1 μg of pGL3-Palb, 0.1 μg of C/EBPs or HNF-1α, and 0.2 μg of CBP and/or p300 expression vectors as indicated (+). In some assays, 0.4 μg of CBP or p300 expression vector was used (++). The bars represent normalized luciferase activity and standard errors for at least three independent experiments.

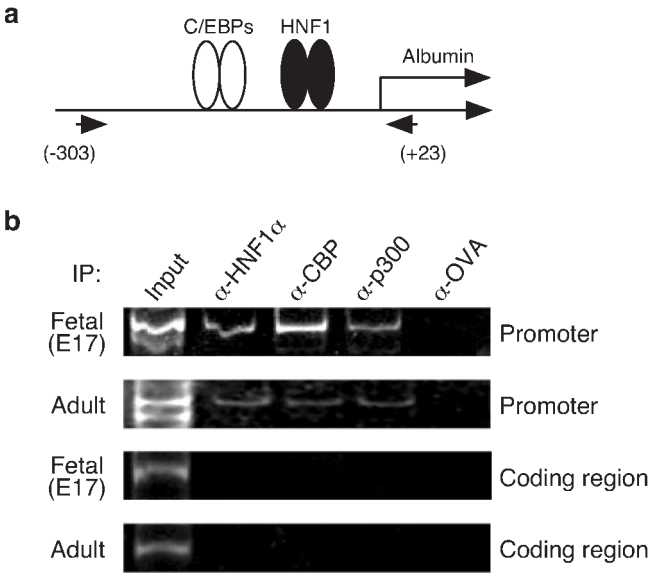

Fig. 4. **Chromatin immunoprecipitation (ChIP) assay of primary hepatocytes with anti-HNF-1α, CBP and p300 antibodies.** (a) Schematic drawing of the albumin promoter region. The positions of PCR primers used for the ChIP assay are shown by arrows at the bottom. (b) ChIP assay for the albumin promoter region using fetal (E17) and adult hepatocytes. The PCR cycle numbers were 34 for fetal hepatocytes and 37 for adult hepatocytes. As negative controls, anti-OVA antibodies were used for immunoprecipitation, and the ChIP assay for the albumin coding region was performed. The input sample (2%) was used as a positive control.

investigate the expression of CBP and p300, primary hepatocytes were isolated from fetal (17 days stage) and adult (6–7 weeks old) rats. The expression of CBP and p300 was analyzed by Western blotting and RT-PCR (Fig. 1a). In adult hepatocytes, the expression of CBP and p300 was low and the amounts of these proteins were under the detection limit for Western blotting. However, on RT-PCR, we could detect low levels of expression of the CBP and p300 genes. The results showed that CBP and p300 were expressed highly in fetal hepatocytes and relatively weakly in adult hepatocytes.

Then, *in vivo* protein-protein interactions between HNF-1α and the coactivators, CBP and p300, were investigated by means of co-immunoprecipitation assays using extracts prepared from fetal hepatocytes. As shown in Fig. 1b, substantial amounts of CBP and p300 were immunoprecipitated with anti-HNF-1α antibodies, indicating that both CBP and p300 interact with HNF-1α in fetal hepatocytes. Furthermore, CBP and p300 were also co-immunoprecipitated from fetal hepatocytes (Fig. 1c). Since the antibodies used in this assay do not cross-react, the result indicated that CBP and p300 coexist in the same protein complex. To confirm these interactions using a model system, we performed co-immunoprecipitation assays with lysates of C33A cells co-transfected with CBP and/or p300 and HNF-1α expression vectors. As shown in Fig. 2a, HNF-1α binds to CBP and p300. CBP and p300 were co-precipitated in the presence and absence of HNF-1α (Fig. 2b). These results also suggested that CBP and p300 are part of the same complex, and that HNF-1α may not mediate the interaction between them.

Both CBP and p300 are Required for Enhanced Transactivation of HNF-1α on the Albumin Promoter—To assess the functional significance of the interaction between CBP and p300, when HNF-1α was expressed together with CBP and/or p300 in SW13 cells, the transcriptional activity of the albumin promoter in the cells was measured using a luciferase reporter assay in which the luciferase gene was controlled by the albumin promoter. SW13 cells were chosen for the assay because they lack endogenous C/EBPα, HNF-1α and ATP-dependent chromatin remodeling factors SWI/SNF. Transfection of CBP and p300 expression vectors resulted in enhanced transactivation of HNF-1α by 1.4-fold and 1.9-fold, respectively. Furthermore, co-expression of CBP and p300 led to 3.8-fold enhancement (Fig. 3). Similar cooperative effects of the coactivators on HNF-1α transactivation were observed in the reporter assay with NIH 3T3 cells (data not shown) lacking endogenous HNF-1α, and expressing the lowest level of endogenous CBP and P/CAF compared to other non-hepatic cell lines (*23*).

Since CBP/p300-dependent transactivation of C/EBPα and C/EBPβ has been reported for several promoters (*15*, *36*), we then studied the effect of CBP/p300 on the transactivation of C/EBPs. As shown in Fig. 3, CBP and p300 had no effect on the transcription mediated by C/EBPα and C/EBPβ. These results suggest that the recruitment of CBP and p300 mediated by HNF-1α occurs at the albumin promoter and that C/EBPs may not aid the recruitment.

Both CBP and p300 Exist in the Albumin Promoter Region in Primary Hepatocytes—To determine whether both CBP and p300 exist in the albumin promoter region in primary hepatocytes, we performed ChIP assays. For the assays, the rat albumin promoter region containing a HNF-1α binding site was amplified by PCR from immunoprecipitates obtained with anti-CBP, p300 or HNF-1α

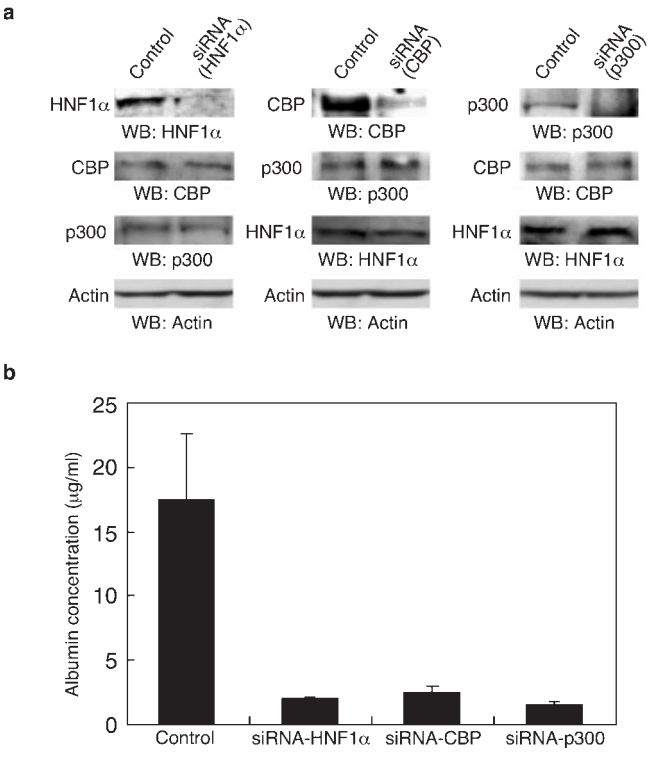

Fig. 5. **Effect of siRNA on albumin expression in primary hepatocyte cultures.** SiRNAs were transfected into fetal (E17) hepatocytes. (a) Expression of HNF-1α, CBP and p300 in the presence of the respective siRNA was analyzed by Western blotting. Western blotting for actin shows normalized protein input. (b) Albumin secretion from hepatocytes transfected with siRNAs was quantified by ELISA.

antibodies (Fig. 4). A specific DNA fragment was amplified for all the samples except for negative controls, and the results suggested that the coactivators and HNF-1α existed in the albumin promoter region.

Both CBP and p300 are Required for Enhanced Albumin Expression in Primary Hepatocytes—To confirm that both CBP and p300 are required for enhanced expression of the albumin gene in hepatocytes, we performed an RNA interference (RNAi) experiment using siRNAs and primary hepatocyte cultures. The siRNAs for HNF-1α, CBP and p300 were designed from the respective mRNA sequences. As shown in Fig. 5a, siRNAs were effectively transfected, and specifically inhibited the expression of HNF-1α, CBP and p300 in fetal hepatocytes. Albumin expression decreased when either CBP or p300 was repressed (Fig. 5b). Similar effects of siRNAs were observed with adult hepatocytes (data not shown). These results indicate that a reduction in either coactivator protein results in decreased albumin expression in hepatocytes. Thus, CBP and p300 may have specific functions and cannot compensate for each other.

DISCUSSION

To date, the function of CBP/p300 has been studied mainly in model systems. Few studies have examined whether the results obtained with model systems apply to physiological systems such as primary cell cultures. In this regard, we analyzed the function of coactivator proteins in albumin gene expression in primary hepatocytes. These proteins have been shown to interact with a wide variety of transcription factors including HNF-1α, coactivators and the general transcription apparatus in the RNA polymerase II preinitiation complex (*17*). Among the transcription factors abundant in liver, HNF-1α was reported to interact with CBP, as found in co-immunoprecipitation experiments in Cos-1 cells over-expressing HNF-1α and CBP as a model system (*23*). In this study, we detected HNF-1α/CBP and HNF-1α/p300 complexes in hepatocytes (Fig. 1b). The ChIP assay also revealed that CBP and p300 were recruited to the albumin promoter region in hepatocytes (Fig. 4). Furthermore, CBP and p300 were co-immunoprecipitated with the proteins extracted from hepatocytes (Fig. 1c), and C33A cells over-expressed these coactivator proteins (Fig. 2b). Although we could not rule out the possibility of direct interaction between CBP and p300, the coactivator proteins may interact through other proteins included in the RNA polymerase II preinitiation complex. Since the co-immunoprecipitation of CBP and p300 was not significantly affected by HNF-1α (Fig. 2b), the HNF-1α dimer seems not to be the adaptor protein for the coactivators. It has been reported that P/CAF potentially interacts with CBP and HNF-1α, and that CBP also interacts with HNF-1α (*23*). Since CBP and p300 exhibit around 90% homology at the P/CAF binding site (*16*), it is possible that P/CAF mediates the interaction between CBP and p300. In addition, the coactivator proteins exhibited cooperative effects on albumin expression in both the luciferase reporter assay and RNAi experiments. These results show the functional importance of the CBP/p300 interaction in HNF-1α-mediated gene transcription.

Although a mechanism for the cooperativity between the coactivator proteins remains unknown, two different mechanisms can be assumed from previous reports. It was reported that p300 and CBP are sequentially recruited to the cathepsin D promoter, this being mediated by estrogen receptor α (*35*). This suggested that CBP and p300 may assemble in different manners and possibly have different functions in the ERα transcriptional complex. These proteins may associate through interaction with some protein in the HNF-1α transcriptional complex on the albumin promoter as well.

Another possibility for the cooperativity might be related to the state of histone acetylation in the albumin promoter region. Since it has been reported that CBP and PCAF interact with each other in the presence of HNF-1α, resulting in enhancement of their HAT activity (*23*, *24*), the possibility of a similar association between CBP and p300 cannot be excluded. It has also been reported that HNF-1α requires the hyperacetylation of nucleosomes at its target gene for its transcriptional activity (*37*). Hyperacetylation in the albumin promoter region due to the cooperative effects of these HAT proteins may be required for the transcriptional activation.

In this study, we used an about 200 bp region of the albumin promoter that contained the C/EBPs binding site in addition to that of HNF-1α. Several studies have indicated that C/EBPs also play important roles in albumin expression (*38*, *39*). Physical interaction between p300 and C/EBPβ has been shown as well (*14*, *15*).

Erickson *et al*. reported functional interaction between C/EBPα and p300 in the activation of the leptin promoter, although they could not detect any physical interaction between these proteins (*36*). Furthermore, it was recently reported that the binding of C/EBPs including C/EBPα to the E1A binding site of p300 triggered massive phosphorylation of p300 (*15*). We studied the effects of CBP and p300 on the transcriptional activity of C/EBPs in the albumin promoter region. However, we could not observe any enhancement of C/EBP-mediated transactivation on the albumin promoter, nor could we detect any physical interaction between C/EBPα and CBP/p300 in hepatocytes (data not shown). On the other hand, ATP-dependent chromatin remodeling factors SWI/SNF interacted with C/EBPα in hepatocytes and activated the albumin promoter, but did not affect HNF-1α–mediated transactivation (Inayoshi *et al*., unpublished results). Therefore, it is speculated that HNF-1α recruits CBP/p300, and C/EBPα interacts with SWI/SNF for sufficient expression of the albumin gene in hepatocytes.

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REFERENCES

1. Chrivia, J.C., Kwok, R.P., Lamb, N., Hagiwara, M., Montminy, M.R., and Goodman, R.H. (1993) Phosphorylated CREB binds specifically to the nuclear protein CBP. *Nature* **365**, 855–859
2. Eckner, R., Ewen, M.E., Newsome, D., Gerdes, M., DeCaprio, J.A., Lawrence, J.B., and Livingston, D.M. (1994) Molecular cloning and functional analysis of the adenovirus E1A-associated 300-kD protein (p300) reveals a protein with properties of a transcriptional adaptor. *Genes Dev.* **8**, 869–884
3. Ogryzko, V.V., Schiltz, R.L., Russanova, V., Howard, B.H., and Nakatani, Y. (1996) The transcriptional coactivators p300 and CBP are histone acetyltransferases. *Cell* **87**, 953–959
4. Chen, H., Lin, R.J., Schiltz, R.L., Chakravarti, D., Nash, A., Nagy, L., Privalsky, M.L., Nakatani, Y., and Evans, R.M. (1997) Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. *Cell* **90**, 569–580
5. Gerritsen, M.E., Williams, A.J., Neish, A.S., Moore, S., Shi, Y., and Collins, T. (1997) CREB-binding protein/p300 are transcriptional coactivators of p65. *Proc. Natl. Acad. Sci. USA* **94**, 2927–2932
6. Kamei, Y., Xu, L., Heinzel, T., Torchia, J., Kurokawa, R., Gloss, B., Lin, S.C., Heyman, R.A., Rose, D.W., Glass, C.K., and Rosenfeld, M.G. (1996) A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptors. *Cell* **85**, 403–414
7. Oelgeschlager, M., Janknecht, R., Krieg, J., Schreek, S., and Luscher, B. (1996) Interaction of the co-activator CBP with Myb proteins: effects on Myb-specific transactivation and on the cooperativity with NF-M. *EMBO J.* **15**, 2771–2780
8. Vries, R.G., Prudenziati, M., Zwartjes, C., Verlaan, M., Kalkhoven, E., and Zantema, A. (2001) A specific lysine in c-Jun is required for transcriptional repression by E1A and is acetylated p300. *EMBO J.* **20**, 6095–6103
9. Martens, J.H., Verlaan, M., Kalkhoven, E., and Zantema, A. (2003) Cascade of distinct histone modifications during collagenase gene activation. *Mol. Cell. Biol.* **23**, 1808–1816
10. Puri, P.L., Avantaggiati, M.L., Balsano, C., Sang, N., Graessmann, A., Giordano, A., and Levrero, M. (1997) p300 is required for MyoD-dependent cell cycle arrest and muscle-specific gene transcription. *EMBO J.* **16**, 369–383
11. Sartorelli, V., Puri, P.L., Hamamori, Y., Ogryzko, V., Chung, G., Nakatani, Y., Wang, J.Y., and Kades, L. (1999) Acetylation of MyoD directed by PCAF is necessary for the execution of the muscle program. *Mol. Cell* **4**, 725–734
12. Gu, W., Shin, X.L., and Roeder, R.G. (1997) Synergistic activation of transcription by CBP and p53. *Nature* **387**, 819–823
13. Lee, C.W., Sorensen, T.S., Shikama, N., and La Thangue, N.B. (1998) Functional interplay between p53 and E2F through co-activator p300. *Oncogene* **16**, 2695–2710
14. Mink, S., Haenig, B., and Klempnauer, K.H. (1997) Interaction and functional collaboration of p300 and C/EBPβ. *Mol. Cell. Biol.* **17**, 6609–6617
15. Schwartz, C., Beck K., Mink, S., Schmolke, M., Budde, B., Wenning, D., and Klempnauer, K.H. (2003) Recruitment of p300 by C/EBPβ triggers phosphorylation of p300 and modulates coactivator activity. *EMBO J.* **22**, 882–892
16. Chan, H.M. and La Thangue, N.B. (2001) p300/CBP proteins: HATs for transcriptional bridges and scaffolds. *J. Cell Sci.* **114**, 2363–2373
17. Cosma, M.P. (2002) Ordered recruitment: gene-specific mechanism of transcription activation. *Mol. Cell* **10**, 227–236
18. Kuo, C.J., Mendel, D.B., Hansen, L.P., and Crabtree, G.R. (1991) Independent regulation of HNF-1 α and HNF-1 β by retinoic acid in F9 teratocarcinoma cells. *EMBO J.* **10**, 2231–2236
19. Maire, P., Wuarin, J., and Schibler, U. (1989) The role of cis-acting promoter elements in tissue-specific albumin gene expression. *Science* **244**, 343–346
20. Schrem, H., Klempnauer, J., and Borlak, J. (2002) Liver-enriched transcription factors in liver function and development. Part I: the hepatocyte nuclear factor network and liver-specific gene expression. *Pharmacol. Rev.* **54**, 129–158
21. Bach, I., Mattei, M.G., Cereghini, S., and Yaniv, M. (1991) Two members of an HNF1 homeoprotein family are expressed in human liver. *Nucleic Acids Res.* **19**, 3553–3559
22. Mendel, D.B., Khavari, P.A., Conley, P.B., Graves, M.K., Hansen, L.P., Admon, A., and Crabtree, G.R. (1991) Characterization of a cofactor that regulates dimerization of a mammalian homeodomain protein. *Science* **254**, 1762–1767
23. Soutoglou, E., Papafotiou, G., Katrakili, N., and Talianidis, I. (2000) Transcriptional activation by hepatocyte nuclear factor-1 requires synergism between multiple coactivator proteins. *J. Biol. Chem.* **275**, 12515–12520
24. Soutoglou, E., Viollet, B., Vaxillaire, M., Yaniv, M., Pontoglio, M., and Talianidis, I. (2001) Transcription factor-dependent regulation of CBP and P/CAF histone acetyltransferase activity. *EMBO J.* **20**, 1984–1992
25. Hamamoto, R., Yamada, K., Kamihira, M., and Iijima, S. (1998) Differentiation and proliferation of primary rat hepatocytes cultured as spheroids. *J. Biochem.* **124**, 972–979
26. Dohda, T., Nakamura, Y., Kamihira, M, and Iijima, S. (2004) Functional role of RhoA in growth regulation of primary hepatocytes. *J. Biochem.* **135**, 631–637
27. Lorenzo, M., Roncero, C., and Benito, M. (1986) The role of prolactin and progesterone in the regulation of lipogenesis in maternal and fetal rat liver *in vivo* and in isolated hepatocytes during the last day of gestation. *Biochem. J.* **239**, 135–139
28. Hamamoto, R., Kamihira, M., and Iijima, S. (1999) Growth and differentiation of cultured fetal hepatocytes isolated from various developmental stages. *Biosci. Biotechnol. Biochem.* **63**, 395–401
29. Laemmli, U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227**, 680–685
30. Landschulz, W.H., Johnson, P.F., Adashi, E.Y., Graves, B.J., and Mcknight, S.L. (1988) Isolation of a recombinant copy of the gene encoding C/EBP. *Genes Dev.* **2**, 786–800
31. Ossipow, V., Descombes, P., and Schibler, U. (1993) CCAAT/enhancer-binding protein mRNA is translated into multiple

proteins with different transcription activation potentials. *Proc. Natl. Acad. Sci. USA* **90**, 8219–8223
32. Miura, N., Iwai, K., and Miyamoto, I. (1993) Immunological characterization of hepatocyte nuclear factor 1 protein: appearance of hepatocyte nuclear factor 1 protein in developing mouse embryos. *Eur. J. Cell Biol.* **60**, 376–382
33. Kwok, R.P., Lundblad, J.R., Chrivia, J.C., Richards, J.P., Bachinger, H.P., Brennan, R.G., Roberts, S.G., Green, M.R., and Goodman, R.H. (1994) Nuclear protein CBP is a coactivator for the transcription factor CREB. *Nature* **370**, 223–226
34. Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Smith, J.A., Seidman, J.G., and Struhl, K. (1987) *Current Protocols in Molecular Biology.* John Wiley and Sons, New York, N.Y.
35. Shang, Y., Hu, X., DiRenzo, J., Lazar, M.A., and Brown, M. (2000) Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. *Cell* **103**, 843–852
36. Erickson, R.L., Hemati, N., Ross, S.E., and MacDouglad, O.A. (2001) p300 coactivates the adipogenic transcription factor CCAAT/enhancer-binding protein α. *J. Biol. Chem.* **276**, 16348–16355
37. Parrizas, M., Maestro, M.A., Boj, S.F., Paniagua, A., Casamitjana, R., Gomis, R., Rivera, F., and Ferrer, J. (2001) Hepatic nuclear factor 1-α directs nucleosomal hyperacetylation to its tissue-specific transcriptional targets. *Mol. Cell. Biol.* **21**, 3234–3243
38. Nerlov, C. and Ziff, E.B. (1994) Three levels of functional interaction determine the activity of CCAAT/enhancer binding protein-alpha on the serum albumin promoter. *Genes Dev.* **8**, 350–362
39. Trautwein, C., Rakemann, T., Pietrangelo, A., Plumpe, J., Montosi, G., and Manns, M.P. (1996) C/EBP-β/LAP controls down-regulation of albumin gene transcription during liver regeneration. *J. Biol. Chem.* **271**, 22262–22270