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The Methyl Transferase PRMT1 Functions as Co-Activator of Farnesoid X Receptor (FXR)/9-*cis* Retinoid X Receptor and Regulates Transcription of FXR Responsive Genes[S]

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ABSTRACT

The farnesoid X receptor (FXR) is a nuclear receptor that functions as an endogenous sensor for bile acids (BAs). FXR is bound to and activated by bile acid, and chenodeoxycholic acid (CDCA) is the natural most active ligand. Upon activation, FXR heterodimerizes with the 9-*cis* retinoic X receptor (RXR) and regulates genes involved in cholesterol and BA homeostasis. 6-Ethyl CDCA (6-ECDCA) is a synthetic BA that binds FXR and induces gene transcription by recruiting coactivators, such as steroid receptor coactivator-1, with histone acetyltransferase activity. In addition to acetylation, histone methylation is critically involved in regulating eukaryotic gene expression. In the present study, we demonstrated that 6-ECDCA activates FXR to interacts with Protein Arginine Methyl-Transferase type I (PRMT1), which induces up-regulation of bile salt export pump (BSEP) and the small heterodimer partner (SHP) mRNA expression and causes a down-regulation of P450 cholesterol 7α-hydroxylase and Na^+ taurocholate cotransport peptide genes. Chromatin immunoprecipitation assay suggests that 6-ECDCA induces both the recruitment of PRMT1 and the H4 methylation to the promoter of BSEP and SHP genes. We also provide evidence that a methyltransferase inhibitor blocks the activation of FXR-responsive genes. Our results indicate that histone methylation, similar to acetylation, regulates transcriptional activation of genes involved in cholesterol and BAs homeostasis.

Bile acids (BAs) regulate their own biosynthesis and transport by binding to and activating the farnesoid X receptor (FXR), a nuclear receptor expressed in liver, intestine, gallbladder, and kidney (Forman et al., 1995; Makishima et al., 1999; Parks et al., 1999; Wang et al., 1999). Chenodeoxycholic acid (CDCA), a primary BA, is the natural most active ligand of FXR, with an EC_{50} of 10 to 50 μM (Makishima et al., 1999; Parks et al., 1999; Wang et al., 1999). We have shown previously that 6α-ethyl-chenodeoxycholic acid (6-ECDCA), a semisynthetic derivative of CDCA, is a potent and selective steroidal FXR agonist with an EC_{50} of 99 nM (Pellicciari et al., 2002) and protects against cholestasis and liver fibrosis when administered in vivo (Fiorucci et al., 2004, 2005).

In liver cells, activation of FXR leads to the regulation of genes whose function is to decrease the concentrations of BAs within hepatocyte. Thus, upon ligand-induced activation, FXR causes a small heterodimer partner (SHP)-dependent inhibition of the expression of P450 cholesterol 7α-hydroxylase (CYP7A1) and oxysterol 12α-hydroxylase (CYP8B1), both of which are central to the synthesis of BAs from cholesterol (Goodwin et al., 2000; Lu et al., 2000; Sinal et al., 2000; del Castillo-Olivares and Gil, 2001; Zhang and Chiang, 2001). In addition, FXR ligands promote the expression of

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ABBREVIATIONS: BA, bile acid; FXR, farnesoid X receptor; CDCA, chenodeoxycholic acid; 6-ECDCA, 6α-ethyl-chenodeoxycholic acid; SHP, small heterodimer partner; BSEP, bile-salt export pump; 9-*cis* RA, 9-*cis*-retinoic acid; RXR, 9-*cis*-retinoic acid receptor; SRC-1, steroid receptor coactivator-1; CARM1, coactivator-associated arginine methyltransferase 1; HMT, histone methyltransferase; SAM, *S*-adenosylmethionine; PRMT, protein arginine methyltransferase; ChIP, chromatin immunoprecipitation assay; RT-PCR, reverse transcription-polymerase chain reaction; GST, glutathione *S*-transferase; HEK, human embryonic kidney; CMV, cytomegalovirus; βgal, β-galactosidase; GW-4064; DMSO, dimethyl sulfoxide; PBS, phosphate-buffered saline; PAGE, polyacrylamide gel electrophoresis; qRT, quantitative real-time; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; cyt *c*, cytochrome *c*; MTA, 5′-deoxy-5′-methylthioadenosine; NTCP, Na^+ taurocholate cotransport peptide; FXRE, farnesoid X receptor response element.

canalicular transporters, such as the bile-salt export pump (BSEP), the multidrug resistance associated protein-2 (Sinal et al., 2000; Ananthanarayanan et al., 2001), and the multidrug resistance protein-2 (Kast et al., 2002), involved in BAs transport across the canalicular membrane of hepatocytes, providing a pathway for cholesterol and BAs excretion.

In liver diseases, accumulation of toxic BAs plays a mechanistic role in hepatocyte injury, leading to cell necrosis, fibrosis, and cirrhosis. Work from our laboratory and others has provided evidence that FXR activation with potent ligands protects against cholestasis (Liu et al., 2003) and liver fibrosis in rodents (Fiorucci et al., 2004, 2005).

FXR is an obligate partner of the 9-*cis*-retinoic acid (9-*cis* RA) receptor (RXR) (Forman et al., 1995; Zavacki et al., 1997; Wang et al., 1999). The FXR/RXR heterodimer binds DNA sequences composed of two inverted repeats separated by one nucleotide (inverted repeat-1) and can be activated by the ligands of both receptors (BAs and/or 9-*cis* RA) (Forman et al., 1995; Zavacki et al., 1997; Wang et al., 1999). There is evidence that 6-ECDCA binds FXR and induces gene transcription by recruiting coactivators with histone acetyltransferase activity, such as the steroid receptor coactivator-1 (SRC-1) (Berger, 2002; Pellicciari et al., 2002).

Histone methylation occurs on lysine or arginine residues and is catalyzed by a family of histone methyltransferases (HMTs) that use *S*-adenosylmethionine (SAM) as a methyl-group donor. Methylation of lysine residues is known to occur on histone H3 (Lys4, Lys9, and Lys27) and H4 (Lys20) (Zhang and Reinberg, 2001; Kouzarides, 2002). Methylation at arginine residues occurs within the tails of histone H3 (Arg2, Arg 17, and Arg 26) and H4 (Arg3) (Strahl et al., 2001; Zhang and Reinberg, 2001; Kouzarides, 2002). Unlike lysine methylation, which exerts a repressive or active function dependent on the promotor context (Zhang and Reinberg, 2001; Kouzarides, 2002; Santos-Rosa et al., 2002), methylation of arginine residues, similar to acetylation, correlates with the active state of transcription (Ma et al., 2001; Bauer et al., 2002). There are five known protein arginine methyltransferases (PRMTs) that have a highly conserved catalytic domain (Kouzarides, 2002). PRMT1, PRMT3, and PRMT4/CARM1 catalyze the formation of asymmetric dimethylated arginine, whereas PRMT5/JBP1 catalyzes symmetric dimethylation. The enzymatic activity of the PRMT2 protein has not yet been established (McBride and Silver, 2001; Zhang and Reinberg, 2001; Kouzarides, 2002). PRMT1 is the predominant, if not exclusive, H4 Arginine-Methyltransferase in mammalian cells and functions as a coactivator of several nuclear receptors, including the thyroid, estrogen (Klinge et al., 2004), and androgen receptors (Strahl and Allis, 2000; Tang et al., 2000; Koh et al., 2001; Strahl et al., 2001). Whether PRMT1 mediates chromatin remodeling in response to FXR ligands is unknown.

In the present study, we demonstrated that FXR immunoprecipitates contained an HMT activity and that FXR bound to PRMT1. Moreover, by chromatin immunoprecipitation assay (ChIP), we provide evidence that natural and synthetic FXR ligands enhanced the interaction between FXR/RXR and PRMT1, leading to the formation of a ternary complex on the promoter of BSEP and SHP, two FXR-responsive genes. Together, these results indicate that histone H4 methylation induced by FXR activation plays a functional role in regulating cholesterol and BA homeostasis.

Materials and Methods

Molecular Cloning of FXRα, RXRα, and PRMT1 and Plasmid Construction. Human cDNAs encoding FXRα, RXRα, and PRMT1 were cloned by RT-PCR from HepG2 cells. In brief, RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions, and 1 μg of RNA was randomly reverse-transcribed with Moloney murine leukemia virus reverse transcriptase (Invitrogen) in a 20-μl reaction volume for 1 h at 42°C. The cDNA was amplified using 250 ng of reverse-transcribed template using Phusion DNA polymerase (FINNzymes, Espoo, Finland) in a 50-μl reaction volume containing 1× Phusion reaction buffer, 200 nM dNTPs, and a 1 μM concentration of each primer. The sequences of the primers used were: FXRα, 5′-tggatgggatcaaaaatgaatctc-3′ and 5′-catcactgcacgtcccagatttc-3′; RXRα, 5′-catgagttagtcgcagacatggac-3′ and 5′-gcctaagtcatttggtgcggcg-3′; and PRMT1, 5′-cgaactgcatcatggaggtgtcctg-3′ and 5′-ctcagcgcatccggtagtcggtg-3′.

The RXR and PRMT1 cDNAs were cloned into EcoRI cloning site and into pSG5 mammalian expression vector, and PRMT1 was cloned into pGEX-4T1 (Amersham Biosciences, Piscataway, NJ) to express the GST fusion protein. The FXR cDNA was cloned into the BglII site in pSG5 (Stratagene, La Jolla, CA). For luciferase assays, the reporter plasmid pGL3(IR1)$_3$-Luc was constructed by cloning three FXREs (IR1) upstream to luciferase into the BglII site of pGL3-Luc vector. The FXRE sequence from BSEP promoter (−70/−43 nucleotides) was 5′-gcccttagggacattgatccttaggcaa-3′ (Ananthanarayanan et al., 2001). The FXRE (IR1) is underlined. Plasmid pGL3-FXREmut-Luc was generated by mutating the FXRE site using the QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, CA) according to the manufacturer's recommendations. The mutated FXRE sequence was 5′-gcccttagaaacattgatttttaggcaa-3′. The mutated FXRE (IR1) is underlined, and the mutations are shown in italic letters.

Cell Culture, Transfection, and Luciferase Assays. HepG2 and HuH7 (two hepatoma cell lines) and human embryonic kidney (HEK) 293T cells were cultured in Earle's minimal essential medium and high-glucose Dulbecco's modified Eagle's medium, respectively, supplemented with 1% penicillin/streptomycin, 1% L-glutamine, and 10% fetal bovine serum (Invitrogen). Cells were grown at 37°C in 5% CO_2. All transfections were performed using the calcium phosphate coprecipitation method in the presence of 25 μM chloroquine. Twenty-four hours before transfection, HEK 293, HepG2, and HuH7 cells were seeded onto six-well plates at a density of 250,000 to 400,000 cells/well. Transient transfections were performed using 500 ng of reporter vector pGL3-(IR1)$_3$-Luc, 200 ng of pCMV-β-gal as internal control for transfection efficiency, and 50 ng of each receptor expression plasmid (pSG5-FXR, pSG5-RXR, or pSG5-PRMT1). The pGEM vector (Promega, Madison, WI) was added to normalize the amounts of DNA transfected in each assay (2.5 μg). At 36 to 48 h after transfection, cells were stimulated with GW-4064 or 6-ECDCA at 1 μM or 20 μM, respectively, for 18 h, all diluted in DMSO. Control cultures received vehicle (0.1% DMSO) alone. Cells were lysed in 100 μl of diluted reporter lysis buffer (Promega) and 2 μl (HEK 293) or 10 μl (HepG2 and HuH7) of cellular lysates were assayed for luciferase activity using the luciferase assay system (Promega). Luminescence was measured using an automated luminometer. Luciferase activities were normalized for transfection efficiencies by dividing the relative light units by β-galactosidase activity expressed from cotransfected pCMV-β-gal. Each data point was the average of triplicate assays and repeated three times.

Immunoprecipitations. Transfected and untransfected cells were induced with FXR ligands or DMSO (vehicle) for 18 h. Cells were first washed three times with ice-cold PBS and lysed by sonication in either radioimmunoprecipitation assay lysis buffer (1× PBS, 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS) for immunoblotting and lysis buffer A (50 mM Tris, pH 8, 150 mM NaCl, 0.5% Nonidet P-40, and 5 mM EDTA) for in vitro methylation. Both media were supplemented with 0.1 mg/ml phenylmethylsulfonyl flu-

oride and 1× Complete protease inhibitor cocktail (Roche Diagnostics, Indianapolis, IN). Lysates were clarified by centrifugation at 13,000*g* for 10 min, and the protein concentration was adjusted to 1 mg/ml. From 1 to 4 mg of total proteins or 10^7 cells were precleared on a rotating wheel for 1 h at 4°C using protein A Sepharose beads (Amersham Biosciences). Immunoprecipitation was performed overnight at 4°C with 1 μg/ml anti-FXRα (Santa Cruz Biotechnology, Santa Cruz, CA) or anti-CD3 as a control antibody in the presence of 10 μl of protein A Sepharose (Amersham Biosciences). The resultant immunoprecipitates were washed five times with 1 ml of lysis buffer and then used for in vitro methylation or immunoblotting.

Histone Purification, in Vitro Methylation, and Fluorography. Histones were purified from the HEK 293 basic cell line using the acid extraction method. In brief, the cells were lysed on ice in 10 mM HEPES, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothreitol, 1.5 mM phenylmethylsulfonyl fluoride, and 0.2 M HCl for 30 min. Acidic proteins were separated from cellular debris by centrifugation and subjected to dialysis in 0.1 M acetic acid and then in H_2O. Proteins were quantified by the Bradford assay (Bio-Rad, Hercules, CA). Immunoprecipitates were incubated for 30 min at 30°C in 40 μl of lysis buffer A supplemented with 0.8 mM *S*-adenosyl-L-[methyl-^{3}H]methionine, [^{3}H]AdoMet (79 Ci/mmol from a 12.6 μM stock solution in dilute HCl/ethanol, 9:1, pH 2.0–2.5; Amersham Biosciences) and purified histones (1 μg). The positive control for methylation was obtained using a GST-PRMT1 fusion protein (0.5 μg) and purified histones (1 μg). Labeled histones were analyzed by SDS-PAGE followed by fluorography. In brief, after electrophoresis, the gel was stained in Coomassie blue for 15 min and then fixed in destaining solution (40% methanol and 10% acetic acid) for 1 h at room temp. The gel was then soaked in a volume of EN3HANCE (PerkinElmer Life and Analytical Sciences, Boston, MA) equivalent to five times the gel volume for 1 h under gentle agitation. The gel was then incubated in water for 30 to 60 min with gentle shaking, after which it was dried and exposed to a Kodak BioMAX film at the −80°C with an intensifying screen.

Western Blot. Immunoprecipitates or cellular extract were resuspended in 2× SDS-sample Laemmli buffer, boiled for 3 min, and separated by SDS-PAGE. The gel was then analyzed by Western blotting with anti-FXRα or anti-RXRα (both from Santa Cruz Biotechnology) or anti-PRMT1 antibodies (Abcam, Cambridge, UK). All blots were developed with horseradish peroxidase-conjugated secondary antibodies using the enhanced chemiluminescence system (Amersham Biosciences).

Chromatin Immunoprecipitation. A ChIP assay was performed according to the manufacturer's protocols (Upstate Biotechnology, Lake Placid, NY) with minor modifications. In brief, HepG2 cells were cross-linked with 1% formaldehyde at room temperature and then the reaction was terminated by the addition of glycine to a final concentration of 0.125 M. Cells were washed in ice-cold PBS and lysed with SDS lysis buffer (1% SDS, 10 mM EDTA, and 50 mM Trip-HCl, pH 8). Cellular lysates were diluted with ChIP dilution buffer, sonicated, and immunoprecipitated with specific antibody: anti-FXR, anti-SHP, and anti-cytochrome *c* from Santa Cruz Biotech (Santa Cruz, CA); anti-PRMT1 (ab3768) from Abcam (Abcam Ltd, Cambridge, UK) and anti-methyl-H4 (Arg3) (07–213) from Upstate Biotechnology. Immunoprecipitates were collected with protein A/G agarose beads (Upstate Biotechnology) and washed sequentially first with a low-salt wash buffer and then a high-salt wash buffer (Upstate Biotechnology) using manufacturer's recommended procedures. DNA was eluted by addition of 1% SDS and 0.1 M NaHCO3, and the cross-linking reactions were reversed by heating the mixture to 65°C overnight. The DNA was recovered from immunoprecipitated material by proteinase K treatment at 65°C for 1 h followed by phenol/chloroform (1:1) extraction, ethanol precipitation and dissolved into 50 μl of water. Two microliters was used for quantitative real-time PCR (qRT-PCR). Five microliters of PCR reactions were extracted after 40 complete cycles for visualization on agarose gels and stained with ethidium bromide.

qRT-PCR. Total RNA was isolated with TRIzol reagent (Invitrogen) from human HepG2 and HuH7 cells stimulated 6-ECDCA (1 μM), GW-4064 (1 μM), or CDCA (20 μM) for 18 h. One microgram of RNA was incubated with DNaseI (Invitrogen) for 15 min at room temperature followed by 95°C for 5 min in the presence of 2.5 mM EDTA. RNA was reverse-transcribed with Superscript III (Invitrogen) with random primers in volume of 20 μl. For real-time PCR, 100 ng of template was used in a 25-μl reaction containing 0.3 μM concentrations of each primer and 12.5 μl of 2× SYBR Green PCR Master Mix (Bio-Rad Laboratories). All reactions were performed in triplicate using the following cycling conditions: 2 min at 95°C, followed by 50 cycles of 95°C for 10 s and 60°C for 30 s using an iCycler iQ instrument (Bio-Rad, Hercules, CA). The mean value of the replicates for each sample was calculated and expressed as cycle threshold (C_T; cycle number at which each PCR reaction reaches a predetermined fluorescence threshold, set within the linear range of all reactions). The amount of gene expression was then calculated as the difference (ΔC_T) between the C_T value of the sample for the target gene and the mean C_T value of that sample for the endogenous control (GAPDH). Relative expression was calculated as the difference ($\Delta\Delta C_T$) between the ΔC_T values of the test and control (wild type) samples for each target gene. The relative level of expression was expressed as $2^{-\Delta\Delta C_T}$. All PCR primers were designed using the PRIMER3-OUTPUT software and published sequence data obtained from the NCBI database. Primers were as shown in Table 1.

Statistical Analysis. Data were analyzed with a two-tailed Student's *t* test using Prism 3 (Graphpad Software, Inc., San Diego, CA). Values are shown as the mean ± S.E. A p value <0.05 was considered statistically significant.

Results

FXR Ligands Induce H4-Specific Methylation Activity. To investigate whether FXR activation by 6-ECDCA leads to the recruitment of HMT activity, HepG2 cells were exposed to 1 μM 6-ECDCA, and anti-FXR immunoprecipitates were incubated with purified core histones in the presence of *S*-adenosyl-L-[methyl-^{3}H]methionine ([^{3}H] SAM), as a methyl group donor for histone methyltransferases. The reactions were then subjected to SDS-PAGE followed by fluorography. As shown in Fig. 1A, HMT activity was detected in anti-FXR immunoprecipitates and was enhanced by incubating the cells with 6-ECDCA (Fig. 1A, lane 6 versus lane 4). In contrast, immunoprecipitates obtained with the unrelated antibody (anti-CD3) showed only a weak HMT activity (Fig. 1A, lanes 3 and 5). In contrast, no HMT activity was detected in immunoprecipitates with protein A Sepharose alone (Fig. 1A, lanes 1 and 2). The HMT activity found in the FXR immunocomplexes efficiently methylates H4, but not H2A, H2B, or H3 (Fig. 1, A and B). Specificity of methylation of H4

TABLE 1
Primers

hBSEP	5′-gggccattgtacgagatcctaa-3′
	5′-tgcaccgtcttttcactttctg-3′
hCYP7A1	5′-caccttgaggacggttccta-3′
	5′-cgatccaaagggcatgtagt-3′
hGAPDH	5′-gacaacagcctcaagatcatcagc-3′
	5′-gtagaggcagggatgatgttctgg-3′
hSHP	5′-ccaatgatagggcgaaagaa-3′
	5′-gctgtctggagtccttctgg-3′
hNTCP	5′-ctgagcgtcatcctggtgttcatg-3′
	5′-ggtcatcacaatgctgaggtt-3′
hBSEP promoter	5′-ctcgtatgtcactgaactgtgctt-3′
	5′-gcactgaacagaattcaaacttt-3′
hSHP promoter	5′-gctggcttcctggcttagc-3′
	5′-cttatcagatgactcaagtg-3′

was confirmed by incubating core histone proteins with a bacterially expressed GST-PRMT1 fusion protein that only methylates histone H4 (Fig. 1A, lane 7). Induction of HMT activity by the 6-ECDCA was due neither to changes in neither the amount of core histone proteins (Fig. 1B) nor to an increased expression of FXR, RXR, and PRMT1 proteins, as shown by the Western blot assay (Fig. 1C). Similar results were obtained with the natural FXR ligand CDCA (20 μM) and with GW-4064 (1 μM), a nonsteroidal FXR ligand (data not shown).

6-ECDCA Induces FXR to Recruit PRMT1. Because PRMT1 is the predominant arginine methyltransferase in mammalian cells (Tang et al., 2000) and selectively methylates Arg 3 of histone H4 (Strahl et al., 2001; Wang et al., 2001), we investigated whether the H4-specific HMT enzymatic activity revealed by the FXR immunoprecipitates was caused by the recruitment of PRMT1 by FXR. To test this hypothesis, lysates obtained from HepG2 cells were immunoprecipitated with anti-FXR and immunoblotted with anti-PRMT1 antibody. FXR activation with natural (CDCA, not shown) and synthetic (6-ECDCA and GW-4064) ligands caused PRMT1 recruitment (Fig. 2, A and B, lane 5 versus 6 and lane 6 versus 7). No interactions were observed in immunoprecipitates obtained with an unrelated antibody (anti-CD3). The exposure of cells to FXR ligands did not change the relative protein expression in total lysates, as shown in Fig. 1C and Fig. 2, A and B, lanes 1 and 2.

PRMT1 Is a Coactivator of the FXR/RXR Heterodimer. To investigate whether PRMT1 functions as an FXR/RXR coactivator, we transiently transfected HEK 293 cells with vector alone; PRMT1; FXR and RXR; or FXR, RXR, and PRMT1 expression vectors. This cell line was chosen because it expresses very low levels of endogenous FXR. The cells were cotransfected with a plasmid containing the luciferase reporter gene under the control of three FXR response elements [FXRE $(IR1)_3$] and the β-gal expression vector (pCMV-β-gal) to normalize the transfection efficiency and then exposed to 6-ECDCA or DMSO (vehicle). Whereas transfecting HEK 293 cells with the PRMT1 expression vector alone had no effect on the gene reporter activity and was insensitive to the addiction of 6-ECDCA (Fig. 3A), the transient expression of the FXR and RXR plasmids resulted in a little enhancement of the gene reporter activity. However, this response was significantly increased by adding 6-ECDCA (1 μM), and cotransfecting PRMT1 caused a further 3.5-fold enhancement of gene reporter activity in the presence of 6-ECDCA (Fig. 3A). The coactivator effect of PRMT1 in cells induced by 6-ECDCA was abrogated when a reporter plasmid containing the mutated FXRE-Luc was employed for transfection (Fig. 3A), indicating that an intact FXRE is required for the PRMT1 promoter induction by FXR ligands. As illustrated in Fig. 3B, the transactivation of FXRE by PRMT1 was modulated in a concentration-dependent manner by the 6-ECDCA with an $EC_{50} \approx 1$ μM ($n = 4$; $P < 0.05$ versus FXR/RXR). In addition, transfection of PRMT1 enhanced the FXR-dependent transactivation of CDCA or GW-4064 (Fig. 3C). The exposure of HEK293 cells to FXR ligands did not change the relative protein expression in total lysates as shown by the Western blot assay (Fig. 3D).

FXR-Dependent Transactivation Enhanced by PRMT1 Is Induced by RXR Ligand 9-*cis* RA. Because FXR functions as a permissive heterodimer with RXR, and previous data have shown that RXR ligands might transactivate FXR (Forman et al., 1995; Zavacki et al., 1997; Wang et al., 1999), we have then examined whether PRMT1 is involved in the FXR transactivation caused by the RXR ligand 9-*cis* RA. HEK 293 cells were cotransfected with FXR,

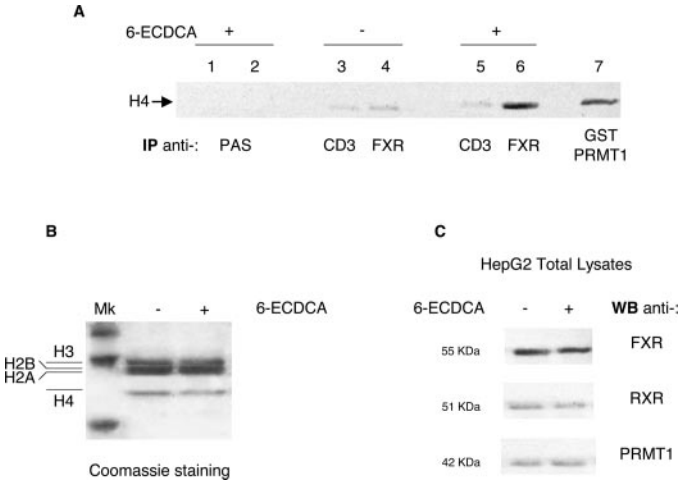

Fig. 1. FXR is associated with H4 HMT activity. A, in vitro methylation. HepG2 cells were induced with or without 1 μM 6-ECDCA, then lysed and immunoprecipitated with a specific anti-FXR antibody or an unrelated antibody (anti-CD3) or protein A Sepharose alone (preclearing). Immunoprecipitates were analyzed for HMT activity in the presence of [^{3}H]SAM, and core histones were purified as described under *Materials and Methods*. Exposure to 6-ECDCA enhances the H4 HMT activity (lanes 4 and 6) in immunoprecipitates obtained with the anti FXR antibody. In contrast, only weak HMT activity was detected in anti-CD3 immunoprecipitates (lanes 3 and 5), whereas no HMT activity was detected in immunoprecipitates with protein A Sepharose (PAS) alone (lanes 1 and 2). Lane 7 is a positive control for in vitro methylation obtained with a GST-PRMT1 fusion protein that selectively methylates histone H4. B, Coomassie blue staining of purified core histones from HepG2 basic cell line indicates that histone protein levels were unmodified by treatment with 6-ECDCA. C, immunoblot of total lysates with anti-FXR, anti-RXR, and anti-PRMT1 antibodies shows that those protein levels were unmodified by 6-ECDCA treatment. Each blot is representative at least two others showing the same pattern.

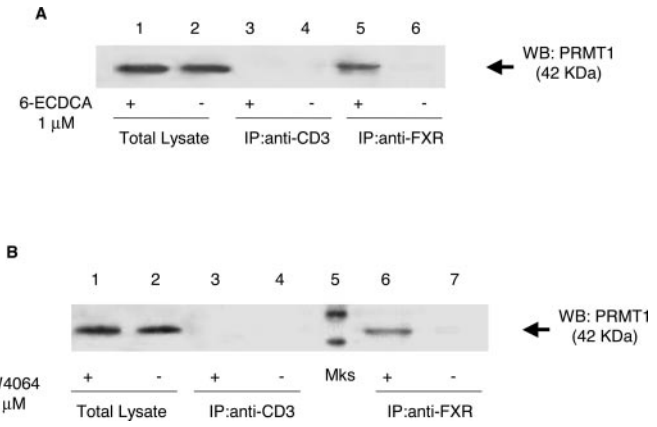

Fig. 2. FXR ligands activate FXR to recruit PRMT1. The cellular extracts from HepG2 cells stimulated with or without to FXR ligands were immunoprecipitated with the anti-FXR or anti-CD3 antibody and then analyzed by Western blotting analysis with anti-PRMT1 antibody. A, 6-ECDCA induces recruitment of PRMT1 to FXR complex. B, a nonsteroidal FXR ligand, GW-4064, induces recruitment of PRMT1. No interaction was detected in anti-CD3 immunoprecipitates (A, lane 1; B, lanes 3 and 4). MKs, protein markers. Each blot is representative at least two others showing the same pattern. The level of the protein PRMT1 was unmodified by treatment of the cells with 6-ECDCA (lanes 1 and 2).

RXR, PRMT1, and the FXRE-Luc reporter gene plasmid and then induced with 1 μM 9-*cis* RA. As shown in Fig. 4, 9-*cis* RA induced a weak transactivation of FXR/RXR heterodimer (Fig. 4, lane 2 versus lane 1). Cotransfection of PRMT1 caused a further enhancement of gene reporter activity in the presence of 9-*cis* RA (Fig. 4, lane 6 versus lane 2), suggesting that the RXR ligand induces the transcriptional activation of FXRE by recruiting a methyltransferase coactivator to the FXR/RXR heterodimer. However, the 9-*cis* RA antagonizes the FXR/RXR transactivation caused by 6-ECDCA (Fig. 4, lane 4 versus lane 3 and lane 8 versus lane 7; $n = 5$, $P < 0.05$ versus 6-ECDCA alone). Similar results were obtained with CDCA and GW-4064 (data not shown).

PRMT1 Enhances Transcriptional Activation of FXR-Responsive Genes Induced by 6-ECDCA. To investigate whether PRMT1 regulates the transcriptional activation of FXR-responsive genes in response to FXR ligands, the HepG2 cell line was transiently transfected with vector alone or PRMT1 plasmid and then stimulated with 6-ECDCA and GW-4064 (data not shown). Figure 5 shows that the relative amounts of BSEP (Fig. 5A) and SHP (Fig. 5B) mRNAs, which are directly regulated by FXR and normalized by GAPDH gene expression, were significantly up-regulated by PRMT1 in the presence of 6-ECDCA ($n = 4$; $P < 0.05$ versus vector alone). The relative expression of NTCP (Fig. 5C) and CYP7A1 (Fig. 5D) was down-regulated by 6-ECDCA ($n = 4$; $P < 0.05$ versus untreated). However, cotransfection of PRMT1 induces a further down-regulation of these two genes induced by the FXR ligand (Fig. 5, C and D) ($n = 4$; $P < 0.05$ versus vector alone). These results correlate with the regulation of SHP, which functions as corepressor for NTCP and CYP7A1 (Goodwin et al., 2000; Lu et al., 2000). Similar results were obtained using HuH7, a human hepatoma cell line (Supplemental Fig. 1).

6-ECDCA Induces Recruitment of PRMT1 to BSEP and SHP Promoters and Histone H4 Methylation. There is evidence that the FXR/RXR heterodimer binds directly to the promoter of FXR-responsive genes (Goodwin et al., 2000; Lu et al., 2000; Ananthanarayanan et al., 2001). In the presence of FXR ligands, FXR/RXR heterodimer recruits coactivators such as SRC-1, which acetylates histones (Berger, 2002; Pellicciari et al., 2002), and CARM1, which methylates histone H3 (Ananthanarayanan et al., 2004). Therefore, we hypothesized that FXR activation might recruit PRMT1 to the promoters of BSEP and SHP. To answer this question, we used the ChiP analysis. A time course

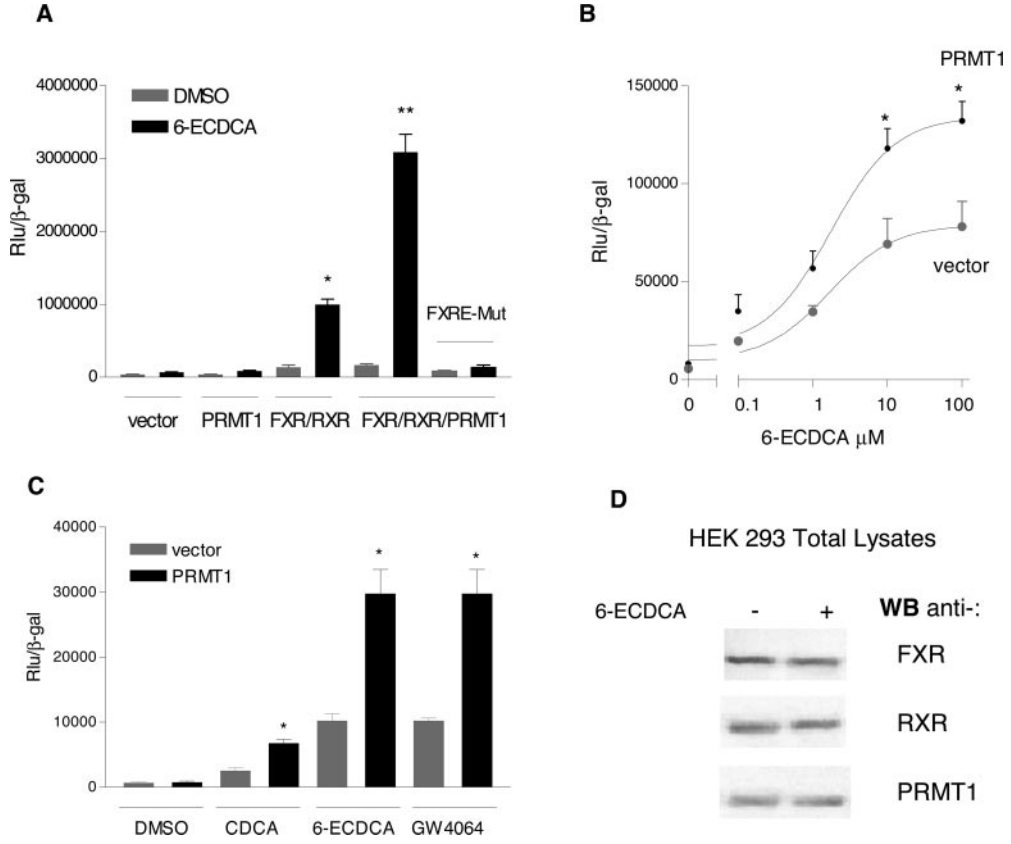

Fig. 3. PRMT1 functions as a coactivator of the FXR/RXR heterodimer. HEK 293 cells were cotransfected with PRMT1, FXR, and RXR expression plasmid, pFXRE-luc reporter plasmid, and pCMV-β-gal plasmid, as control of transfection efficacy. At 48 h after transfection, the cells were induced with DMSO, 1 μM 6-ECDCA, 20 μM CDCA, or 1 μM GW-4064 for a further 18 h. Relative luciferase expression units were measured and normalized by β-gal expression. A, cotransfection of the cells with PRMT1, FXR, and RXR enhances transactivation induced by FXR/RXR in the presence 1 μM 6-ECDCA. The transactivation induced by PRMT1 was almost abrogated by using a pFXRE-luc reporter plasmid with the FXRE mutated (FXRE-Mut). Transfection of PRMT1 alone does not induce transactivation of FXRE, in the presence or absence of 6-ECDCA, indicating that PRMT1 has no intrinsic coactivation function. Data are the mean ± S.E. of four separate experiments. *, $P < 0.05$ versus DMSO alone. **, $P < 0.01$ versus FXR/RXR in the presence of 6-ECDCA. B, 6-ECDCA regulates transactivation induced by PRMT1 in a concentration-dependent manner. Data are the mean ± S.E. of four separate experiments. *, $P < 0.05$ versus FXR/RXR alone. C, PRMT1 enhances FXR/RXR induced transactivation caused by 20 μM CDCA and 1 μM GW-4064. Data are the mean ± S.E. of four separate experiments. *, $P < 0.05$ versus FXR/RXR alone. D, the level of the proteins FXR, RXR, and PRMT1 was unmodified by 6-ECDCA.

analysis of ChIP using the anti–PRMT1 antibody shown in Fig. 6A demonstrates that, in response to 6-ECDCA, occupation of BSEP (and SHP) promoter by PRMT1 peaks at 20 min, and we used this time-frame for all the following experiments ($n = 4$; $P < 0.05$ versus baseline). To investigate whether H4 histone methylation occurred in the BSEP and SHP promoters, the chromatin was prepared from HepG2 cells treated with 6-ECDCA for 20 min and immunoprecipitated with anti-FXR, anti-PRMT1, anti-methyl H4 (Arg 3), or anti-cyt *c* antibodies (control). The qRT-PCR analysis shown in Fig. 6B demonstrates that the 6-ECDCA induces recruitment of PRMT1 to BSEP and SHP promoters and increases H4 arginine methylation. Together, our results provide firm evidence that 6-ECDCA activates FXR to recruit PRMT1 and H4 histone methylation, as a mechanism of transcriptional activation.

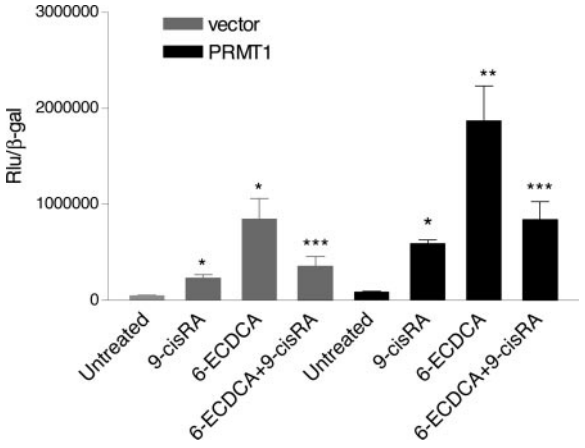

Fig. 4. The RXR ligand 9-*cis* RA enhances FXR/RXR transactivation induced by PRMT1. HEK 293 cells were cotransfected with FXR, RXR, PRMT1, and pGL3(IR1)$_3$-Luc expression plasmids. At 36 h after transfection, the cells were induced for 18 h with DMSO (untreated), 1 μM 6-ECDCA, or 1 μM 9-*cis* RA alone or in combination. Luciferase activities were normalized to the expression of β-gal. The 9-*cis* RA induces enhancement of gene reporter activity in the cells cotransfected with PRMT1, suggesting that the RXR ligand induces a transcriptional activation of FXRE by recruiting a methyltransferase coactivator to the FXR/RXR heterodimer. However, 9-*cis* RA antagonizes the FXR/RXR transactivation caused by 6-ECDCA. Data are the mean ± S.E. of four separate experiments. *, $P < 0.05$ versus untreated cells. **, $p < 0.05$ FXR/RXR alone. ***, $P < 0.05$ versus 6-ECDCA alone.

Inhibition of Arginine Methyltransferase Activity Decreases FXR-Mediated Transcription. Because these data supported a role for arginine methylation in FXR function, we have then investigated the potential effects of the 5′-deoxy-5′-methylthioadenosine (MTA), a methylation inhibitor, on the ability of FXR to induce gene transcription. HepG2 cells were pretreated with 0.3 mM MTA before incubation with 6-ECDCA or GW-4064. The expression of BSEP gene, normalized by GAPDH gene expression, was up-regulated 84- and 89-fold by 6-ECDCA and GW-4064, respectively (Fig. 7A, columns 3 and 4). Exposing the cells to MTA reduced the expression of BSEP induced by either 6-ECDCA or GW-4064 by ~ 80% (Fig. 7A, columns 5 and 6). The same results, with different values, were obtained with SHP gene expression (Fig. 7B).

Discussion

Chromatin remodeling induced by FXR/RXR heterodimer is mediated by histone acetylation. Previous studies have shown that upon FXR activation, SRC-1, a member of p160 family of acetyltransferases, is recruited to the DNA (Berger, 2002; Pellicciari et al., 2002; Mi et al., 2003). Histone acetylation, in concert with H3 and H4 histone methylation, recruits different proteins or protein complexes and regulates diverse chromatin functions

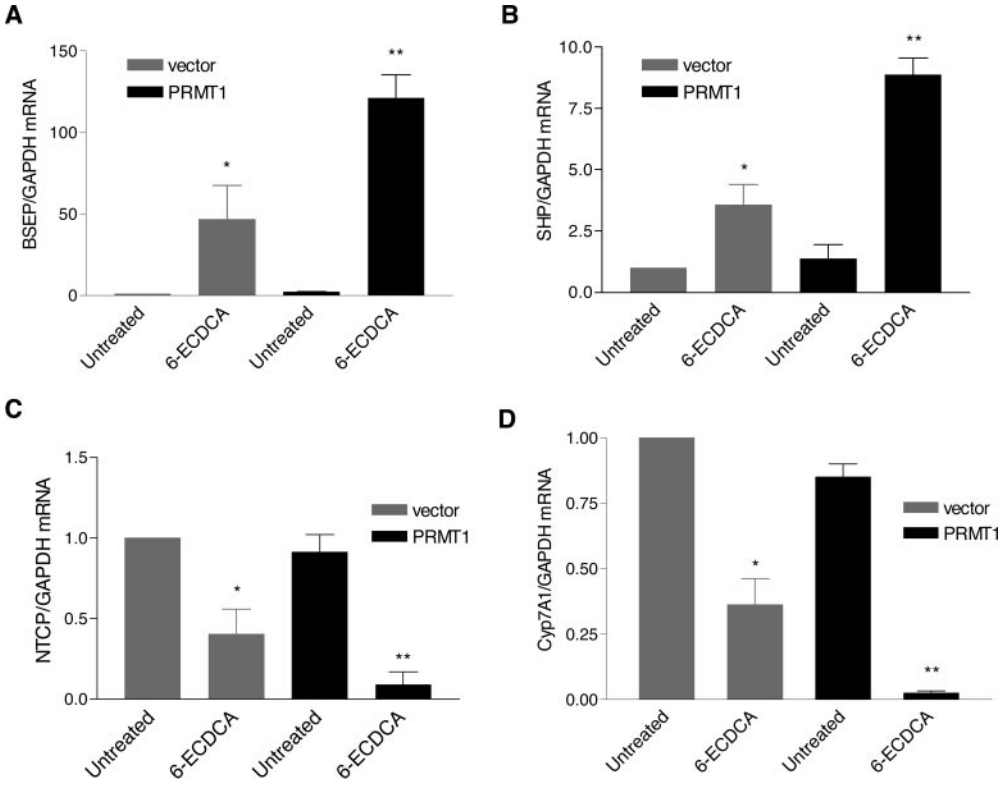

Fig. 5. PRMT1 induces transcriptional activation of FXR-responsive genes. HepG2 cells were transiently transfected with vector alone or PRMT1 expression plasmid and induced with DMSO (untreated) or 1 μM 6-ECDCA. Total RNA was isolated and the relative expression of the FXR responsive genes, normalized by the expression of GAPDH, was evaluated by qRT-PCR. BSEP (A) and SHP (B) mRNA expression was up-regulated by cotransfection of PRMT1 in the presence of 6-ECDCA. NTCP (C) and Cyp7A1 (D) mRNA expression was down-regulated by cotransfection of PRMT1 in the presence of 6-ECDCA. The down-regulation of the NTCP and Cyp7A1 correlates with up-regulation of SHP. Data are the mean ± S.E. of four experiments. *, $P < 0.05$ versus untreated. **, $P < 0.05$ versus vector alone.

(Strahl and Allis, 2000; Zhang and Reinberg, 2001; Kouzarides, 2002). In the present study, using immunoprecipitation and in vitro methylation assays, we demonstrated that in addition to histone acetylation, activation of FXR induces H4 histone methylation. The PRMT1 is the predominant histone methyltransferase in mammalian cells (Strahl and Allis, 2000; Tang et al., 2000; Strahl et al., 2001) and specifically methylates Arginine 3 on H4 histone both in vitro and in vivo (Strahl et al., 2001; Wang et al., 2001). Our immunoprecipitation and Western blot analysis indicate that exposure to natural and synthetic FXR ligands induces the interaction of FXR with PRMT1. This methyltransferase acts as a coactivator for several nuclear receptors, including estrogen, thyroid hormone, and androgen receptors in transient transfection assays, suggesting that nuclear receptors causeschromatin remodeling by inducing histone methylation in addition to histone acetylation (Koh et al., 2001; Strahl et al., 2001; Wang et al., 2001). With the use of luciferase assays, we also demonstrated that PRMT1 increases the transactivation of the luciferase reporter gene induced by FXR and that this function was dependent on an intact FXRE. Finally, we provide evidence that the increase of the FXR-induced transcriptional activation by PRMT1 was dependent on the concentration of FXR ligands.

The FXR/RXR complex is a permissive heterodimer that might be activated by BAs as well as by the RXR ligand 9-*cis* RA (Forman et al., 1995; Zavacki et al., 1997; Wang et al., 1999). An important finding of this study was the demonstration that exposure to the 9-*cis* RA activates the FXR/RXR complex and enhances the transactivation induced by PRMT1. These results suggest that in addition to histone acetylation, transcriptional activation mediated by 9-*cis* RA involves histones methyltransferase and that this pathway uses PRMT1 as a coactivator. Although the 9-*cis* RA activates the FXR/RXR complex, the functional consequence of this interaction is not completely understood. Thus, Kassam et al. (2003) have recently shown that exposure of FXR/RXR heterodimer to 9-*cis* RA decreases, rather than activates, the recruitment of coactivators, as well as the DNA binding activity in the presence of FXR ligands. Consistent with this finding, we demonstrated that 9-*cis* RA antagonizes the transcriptional activation induced by 6-ECDCA. Although the mechanism(s) involved in this antagonism was not investigated, at least two explanations warrant consideration. First, it might be possible that 9-*cis* RA induces the formation of RXR homodimers that bind to the RXR response element

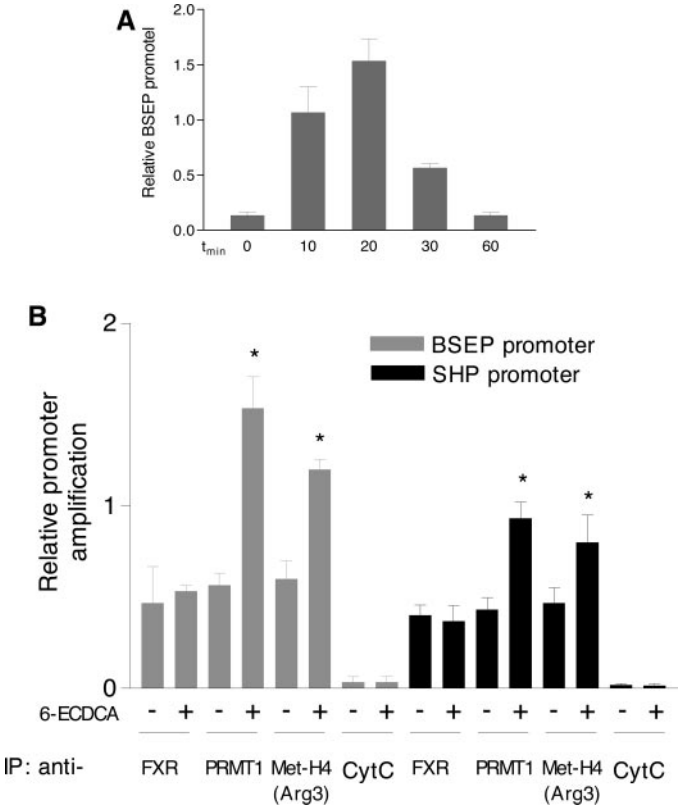

Fig. 6. ChIP analysis. 6-ECDCA induces recruitment of PRMT1 and increases H4 arginine methylation to promoters of FXR responsive genes. Chromatin was prepared as described under *Materials and Methods* and immunoprecipitated with anti-FXR, anti-PRMT1, anti-methyl H4 (Arg3) antibodies, or anti-cyt *c*, as unrelated antibody. A, quantitative PCR analyses of the BSEP promoter in the presence of 6-ECDCA 1 μM at different induction times. The chromatin was prepared from HepG2 cell line, and ChIP analysis was performed with anti-PRMT1 antibody and anti-cyt *c* antibody as negative control (data not shown). B, quantitative PCR shows that the 6-ECDCA induces recruitment of PRMT1 to BSEP and SHP promoters and increases histone H4 arginine methylation. The histograms represent real-time PCR values of promoter amplification [total DNA detected with anti-FXR, anti-PRMT1 or anti-methyl H4 (Arg3) specific antibody minus the DNA quantity detected with the anti-cyt *c* antibody]. Data are the mean ± S.E. of four experiments. *, $P < 0.05$ versus untreated. **, $P < 0.05$ versus vector alone.

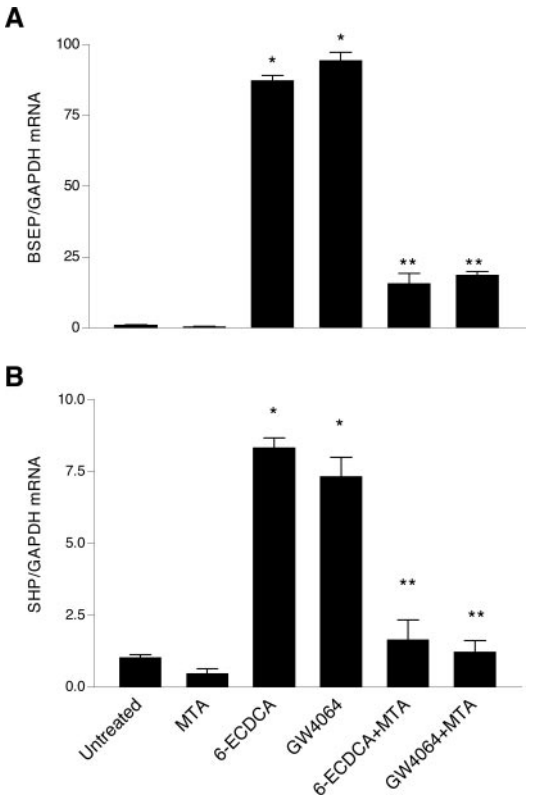

Fig. 7. The inhibitor of protein methylation, MTA, inhibits transcriptional activation of the FXR-responsive genes BSEP and SHP. HepG2 cells were exposed to DMSO (untreated), 1 μM 6-ECDCA, GW-4064, with or without 0.3 mM MTA. After 18 h, total RNA was extracted to evaluate the BSEP and SHP gene expression by qRT-PCR. MTA significantly down-regulates the expression of the BSEP (A) and SHP (B) gene induced by 6-ECDCA and GW-4064. *, $P < 0.001$ versus untreated cells. **, $P < 0.01$ versus FXR ligands.

(Zhang et al., 1992). On the other hand, 9-*cis* RA might induce the heterodimerization of RXR with other partners, such as peroxisome proliferator-activated receptors, RAR and thyroid receptor, thus reducing the availability of RXR for dimerization with FXR (Zhang et al., 1992; Thompson et al., 1998).

In this study, we showed that PRMT1 functions in the transcriptional regulation of FXR-responsive genes. By real-time PCR, we demonstrated that BSEP and SHP, two FXR-regulated genes, were induced by transient transfection of PRMT1 in hepatic cell lines in the presence of FXR ligands. Furthermore, NTCP and CYP7A1, which are direct targets of SHP, were also down-regulated by PRMT1 cotransfection. These effects correlate with the ability of FXR ligands to increase SHP expression in target cells, which directly represses CYP7A1 and NTCP (Goodwin et al., 2000; Lu et al., 2000).

BSEP is the major BA exporting pump and plays an integral role in lipid homeostasis by regulating the canalicular excretion of BAs. Indeed, inactivating mutations of this gene result in progressive familial intrahepatic cholestasis (type 2) and liver cirrhosis (Strautnieks et al., 1998). In the presence of FXR ligands, the FXR/RXR heterodimer binds to an FXRE located in the BSEP promoters and induces the transcriptional activation of BSEP (Ananthanarayanan et al., 2001). The results obtained by ChIP assay suggest that 6-ECDCA induces both the recruitment of PRMT1 and H4 histone methylation to the BSEP promoter. Similar results were obtained with another FXR-regulated gene (i.e., SHP, which is a master regulator of several nuclear receptors). The histone methylation was required to activate gene transcription upon FXR activation. Indeed, we found that exposure to MTA, a methyltransferase inhibitor (Williams-Ashman et al., 1982), antagonizes BSEP and SHP mRNA induction by 6-ECDCA or GW-4064 in HepG2 cells. These results support the notion that activation of FXR-regulated genes induced by FXR ligands requires protein methylation.

In conclusion, we have provided evidence that chromatin remodeling induced by FXR ligands requires H4 histone methylation and that PRMT1 functions as FXR coactivator.

References

Ananthanarayanan M, Balasubramanian N, Makishima M, Mangelsdorf DJ, and Suchy FJ (2001) Human bile salt export pump promoter is transactivated by the farnesoid X receptor/bile acid receptor. *J Biol Chem* **276:**28857–28865.

Bauer UM, Daujat S, Nielsen SJ, Nightingale K, and Kouzarides T (2002) Methylation at arginine 17 of histone H3 is linked to gene activation. *EMBO (Eur Mol Biol Organ) Rep* **3:**39–44.

Berger SL (2002) Histone modifications in transcriptional regulation. *Curr Opin Genet Dev* **12:**142–148.

del Castillo-Olivares A and Gil G (2001) Suppression of sterol 12alpha-hydroxylase transcription by the short heterodimer partner: insights into the repression mechanism. *Nucleic Acids Res* **29:**4035–4042.

Fiorucci S, Antonelli E, Rizzo G, Renga B, Mencarelli A, Riccardi L, Orlandi S, Pellicciari R, and Morelli A (2004) The nuclear receptor SHP mediates inhibition of hepatic stellate cells by FXR and protects against liver fibrosis. *Gatsroenterology* **127:**1497–1512.

Fiorucci S, Clerici C, Antonelli E, Orlandi S, Goodwin B, Sadeghpour BM, Sabatino G, Russo G, Castellani D, Willson TM, et al. (2005) Protective effects of 6-ethyl chenodeoxycholic acid, a farnesoid X receptor ligand, in estrogen-induced cholestasis. *J Pharmacol Exp Ther* **313:**604–612.

Forman B, Goode E, Chen J, Oro AE, Bradley DJ, Perlmann T, Noonan DJ, Burka LT, McMorris T, Lamph WW, et al. (1995) Identification of a nuclear receptor that is activated by farnesol metabolites. *Cell* **81:**687–693.

Goodwin B, Jones SA, Price RR, Watson MA, McKee DD, Moore LB, Galardi C, Wilson JG, Lewis MC, Roth ME, et al. (2000) A regulatory cascade of the nuclear receptors FXR, SHP-1 and LRH-1 represses bile acid biosynthesis. *Mol Cell* **6:**517–526.

Kassam A, Miao B, Young PR, and Mukherjee R (2003) Retinoid X receptor (RXR) agonist-induced antagonism of farnesoid X receptor (FXR) activity due to absence of coactivator recruitment and decreased DNA binding. *J Biol Chem* **278:**10028–10032.

Kast HR, Goodwin B, Tarr PT, Jones SA, Anisfeld AM, Stoltz CM, Tontonoz P, Kliewer S, Willson TM, and Edwards PA (2002) Regulation of multidrug resistance-associated protein 2 (ABCC2) by the nuclear receptors pregnane X receptor, farnesoid X-activated receptor and constitutive androstane receptor. *J Biol Chem* **277:**2908–2915.

Klinge CM, Jernigan SC, Mattingly KA, Risinger KE, and Zhang J (2004) Estrogen response element-dependent regulation of transcriptional activation of estrogen receptors alpha and beta by coactivators and corepressors. *J Mol Endocrinol* **33:**387–410.

Koh SS, Chen D, Lee YH, and Stallcup MR (2001) Synergistic enhancement of nuclear receptor function by p160 coactivators and two coactivators with protein methyltransferase activities. *J Biol Chem* **276:**1089–1098.

Kouzarides T (2002) Histone methylation in transcriptional control. *Curr Opin Genet Dev* **12:**198–209.

Liu Y, Binz J, Numerick MJ, Dennis S, Luo G, Desai B, MacKenzie KI, Mansfield TA, Kliewer SA, Goodwin B, et al. (2003) Hepatoprotection by the farnesoid X receptor agonist GW-4064 in rat models of intra- and extrahepatic cholestasis. *JCI Online* **112:**1678–1687.

Lu TT, Makishima M, Repa JJ, Schoonjans K, Kerr TA, Auwerx J, and Mangelsdorf DJ (2000) Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. *Mol Cell* **6:**507–515.

Ma H, Bauman CT, Li IH, Strahl BD, Rice R, Jelnek MA, Aswad DW, Allis CD, Hager GL, and Stallcup MR (2001) Hormone-dependent, CARM1-directed arginine-specific methylation of histone H3 on a steroid regulated promoter. *Curr Biol* **11:**1981–1985.

Makishima M, Okamoto AY, Repa JJ, Tu H, Learned RM, Luk A, Hull MV, Lustig KD, Mangelsdorf DJ, and Shan B (1999) Identification of a nuclear receptor for bile acids. *Science (Wash DC)* **284:**1362–1365.

McBride AE and Silver PA (2001) State of the Arg: protein methylation at arginine comes of age. *Cell* **106:**5–8.

Mi L, Devarakonda S, Harp JM, Han Q, Pellicciari R, Willson TM, Khorasanizadeh S, and Rastinejad F (2003) Structural basis for bile acid binding and activation of the nuclear receptor FXR. *Mol Cell* **11:**1093–1100.

Parks DJ, Blanchard SG, Bledsoe RK, Chandra G, Consler TG, Kliewer SA, Stimmel JB, Willson TM, Zavacki AM, Moore DD, et al. (1999) Bile acids: natural ligands for an orphan nuclear receptor. *Science (Wash DC)* **284:**1365–1368.

Pellicciari R, Fiorucci S, Camaioni E, Clerici C, Costantino G, Maloney PR, Morelli A, Parks DJ, and Willson TM (2002) 6alpha-ethyl-chenodeoxycholic acid (6-ECDCA), a potent and selective FXR agonist endowed with anticholestatic activity. *J Med Chem* **45:**3569–3572.

Santos-Rosa H, Schneider R, Bannister AJ, Sherriff J, Bernstein BE, Emre NC, Schreiber SL, Mellor J, and Kouzarides T (2002) Active genes are trimethylated at K4 of histone H3. *Nature (Lond)* **419:**407–411.

Sinal CJ, Tohkin M, Miyata M, Ward JM, Lambert G, and Gonzalez FJ (2000) Targeted disruption of the nuclear receptor FXR/BAR impairs bile acid and lipid homeostasis. *Cell* **102:**731–744.

Strahl BD and Allis CD (2000) The language of covalent histone modifications. *Nature (Lond)* **403:**41–45.

Strahl BD, Briggs SD, Brame CJ, Caldwell JA, Koh SS, Ma H, Cook RG, Shabanowitz J, Hunt DF, Stallcup MR, et al. (2001) Methylation of histone H4 at arginine 3 occurs in vivo and is mediated by the nuclear receptor coactivator PRMT1. *Curr Biol* **11:**996–1000.

Strautnieks SS, Bull LN, Knisely AS, Kocoshis SA, Dahl N, Arnell H, Sokal E, Dahan K, Childs S, Ling V, et al. (1998) A gene encoding a liver-specific ABC transporter is mutated in progressive familial intrahepatic cholestasis. *Nat Genet* **20:**233–238.

Tang J, Frankel A, Cook RJ, Kim S, Paik WK, Williams KR, Clarke S, and Herschman HR (2000) PRMT1 is the predominant type I protein arginine methyltransferase in mammalian cells. *J Biol Chem* **275:**7723–7730.

Thompson PD, Jurutka PW, Haussler CA, Whitfield GK, and Haussler M (1998) Heterodimeric DNA binding by the vitamin D receptor and retinoid X receptors is enhanced by 1,25-dihydroxyvitamin D3 and inhibited by 9-*cis*-retinoic acid. Evidence for allosteric receptor interactions. *J Biol Chem* **273:**8483–8491.

Wang H, Chen J, Hollister K, Sowers LC, and Forman BM (1999) Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. *Mol Cell* **3:**543–553.

Wang H, Huang ZQ, Xia L, Feng Q, Erdjument-Bromage H, Strahl BD, Briggs SD, Allis CD, Wong J, Tempst P, et al. (2001) Methylation of histone H4 at arginine 3 facilitating transcriptional activation by nuclear hormone receptor. *Science (Wash DC)* **293:**853–857.

Williams-Ashman HG, Seidenfeld J, and Galletti P (1982) Trends in the biochemical pharmacology of 5′-deoxy-5′-methylthioadenosine. *Biochem Pharmacol* **31:**277–288.

Zavacki AM, Lehmann JM, Seol W, Willson TM, Kliewer SA, and Moore DD (1997) Activation of the orphan receptor RIP14 by retinoids. *Proc Natl Acad Sci USA* **94:**7909–7914.

Zhang M and Chiang JY (2001) Transcriptional regulation of the human sterol 12α-hydroxylase gene (CYP8B1): roles of hepatocyte nuclear factor 4α in mediating bile acid repression. *J Biol Chem* **276:**41690–41699.

Zhang XK, Hoffmann B, Tran PB, Graupner G, and Pfhal M (1992) Retinoid X receptor is an auxiliary protein for thyroid hormone and retinoic acid receptors. *Nature (Lond)* **355:**441–446.

Zhang Y and Reinberg D (2001) Transcription regulation by histone methylation: Interplay between different covalent modifications of the core histone tails. *Genes Dev* **15:**2343–2360.

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