TRANSCRIPTIONAL REGULATION OF PREGNANE X RECEPTOR BY PROTEIN ARGININE METHYLTRANSFERASE

A Dissertation

by

YING XIE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Major Subject: Toxicology

TRANSCRIPTIONAL REGULATION OF PREGNANE X RECEPTOR BY PROTEIN ARGININE METHYLTRANSFERASE

A Dissertation

by

YING XIE

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

Approved by:

Chair of Committee,	Yanan Tian
Committee Members,	Stephen H. Safe
	Robert C. Burghardt
	Beiyan Zhou
	Mark T. Bedford
Head of Department,	Robert C. Burghardt

Major Subject: Toxicology

ABSTRACT

Transcriptional Regulation of Pregnane X Receptor by Protein Arginine Methyltransferase.

Ying Xie, B.S., Peking University

Chair of Advisory Committee: Dr. Yanan Tian

Pregnane X receptor (PXR) is a ligand-dependent transcription factor that plays an important role in xenobiotic/drug metabolism. The ligand-receptor interaction transcriptionally activates phase I and phase II enzymes, and membrane-bound transporters in a coordinated manner and ultimately leads to detoxification and excretion of the ligands. One of the direct target genes is cytochrome P450 3A4 (CYP3A4) which is responsible for metabolism of over 50% of clinically used drugs. Understanding the regulation of PXR is important for treatment of disease and avoidance of untoward drug-drug interactions.

In this research, we have used various biochemical and molecular approaches to investigate factors that regulate the transcriptional activity of PXR. We have stably transfected PXR into HepG2 human liver hepatoma cells. Using these PXR-HepG2 cells, we surveyed the histone methyltransferases that interact with PXR. Based on results from co-immunoprecipitation/methyltransferase, N-terminal peptide sequencing, GST-pulldown assays, we found that protein arginine methyltransferase 1 (PRMT1) is a predominant histone methyltransferase in HepG2 cells.

Evidence from other laboratories suggests that histone methylation by PRMT1 sets the stage for subsequent histone modifications such as the acetylation of histone H4. These modifications are believed to be important for transcriptional and epigenetic regulation of gene expression. We hypothesize that PRMT1 plays a role in the epigenetic changes regulated by PXR. PRMT1-dependent histone methylation changes may be involved in epigenetic cell memory where prior exposure to certain agents may alter the chromatin (or priming the chromatin) with a "primed" state which alters the subsequent magnitude or duration of gene expression.

In our study, we have found that pretreatment of PXR-HepG2 cells with DMSO greatly enhanced PXR-mediated activation of CYP3A4 upon rifampicin treatment. DMSO pretreatment altered histone modifications association with the promoter of the PXR-regulated gene (CYP3A4). Inhibition of histone methylation by PRMT1 either through RNAi or the methyltransferase inhibitor (Adox) abolished the priming effects.

My research results strongly indicate that PRMT1 is involved in transcriptional regulation of PXR and may be involved in epigenetic memory of liver cells where prior exposure to agents changes the subsequent detoxification responses.

ACKNOWLEDGEMENTS

I would like to thank my committee chair, Dr. Yanan Tian, for great guidance and support through my study. I really appreciate his advice on my study of epigenetics, an area I enjoyed learning. I also thank my committee members, Dr. Stephen H. Safe, Dr. Robert C. Burghardt, Dr. Beiyan Zhou, and Dr. Mark T. Bedford for their guidance and support throughout the course of this research. Dr. Kirby C. Donnelly also gave me much encouragement and support in my study. His faithful testimony gave me great encouragement for my future life.

I also would like to thank Sui Ke for great instruction and help with both experiments and life. I appreciate all the members in Dr. Tian's lab for discussion and help with each other, Nengtai Ouyang, Xingsheng Gu, Dr. Duan Liu, Navada Eagleton, Herui Yao, Hongmei Cui, Katy Caraway, Zhixian Yang, Wanjun Zhang, Qiulin Tan and Timothy Yu. I also appreciate the good interaction and kind help from members of our toxicology program. Thanks go to Lorrie Davidson for assistance in real-time PCR.

Thanks also go to Kim Daniel, Kathy Mooney and Cathy Green for their administrative help, which made my time in our toxicology program a great experience.

I also want to thank my parents and all my friends for their encouragement and love. Finally, thanks to all who have instructed, encouraged, motivated and helped me during my studies at Texas A&M University.

TABLE OF CONTENTS

CHAPTER Page

CHAPTER Page

LIST OF FIGURES

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION

1.1 NUCLEAR RECEPTOR

1.1.1 *Nuclear receptor subfamilies*

Nuclear receptors are a superfamily of ligand-activated transcription factors that regulate important physiological functions, such as drug metabolism, development, cell differentiation, and reproduction (1-5) . They are specific for animals, but not in other species (6). There are 270 nuclear receptors in C. elegans (7), while in human, there are only 48 (8).

Evans divided nuclear receptors into three classes based on their dimerization and DNA binding properties (9). Class I includes the steroid hormone receptors, including progesterone receptor (PR), estrogen receptor (ER), glucocorticoid receptor (GR), androgen receptor (AR), and the mineralocorticoid receptor (MR). Upon ligand binding, they dissociate from heat shock proteins, translocate into the nucleus, and bind cognate response elements as homodimers. Response elements for class I receptors are always inverted repeats, for example, 5′-AGAACA*nnn*TGTTCT-3′ (10).

The thyroid/retinoid receptors, including the thyroid receptor (TR), vitamin D receptor (VDR), retinoic acid receptor (RAR), and the peroxisome proliferator activated receptors (PPARs) are the second class of nuclear receptors (11). Thyroid/retinoid

---

This dissertation follows the style of *Journal of Biological Chemistry*.

receptors typically bind as heterodimers with RXR to direct repeats, such as AGGTCA$(N)_4$AGGTCA (12). In the absence of agonist, corepressor proteins bind the heterodimers to prevent gene expression. After agonist binding, corepressor proteins dissociate from the receptor, and coactivators are recruited for activation of transcription (13,14).

The third class orphan nuclear receptors typically have no known endogenous ligands at time of their identification (15,16). Recent studies have identified ligands for several orphan receptors, and these are adopted orphan receptors, which include liver X receptor (LXR), pregnane X receptor (PXR), and farnesol X receptor (FXR) (17-19). Some orphan nuclear receptors bind response elements as heterodimers with RXR (20), and some bind as monomers or homodimers. The everted repeat, such as TGAACT$(N)_6$AGGTCA, is another motif recognized by these receptors (21), and Table 1.1 lists the ligand, expressing tissue, response elements and target genes for some nuclear receptors.

The nuclear receptors are also divided 7 subfamilies for their formal nomenclature; subfamily 0-6, and listed in Table1.2 which follows (22).

Table 1.1. Nuclear Receptors – ligand, response element, target genes

NR	Ligands	Response element	Tissue	Target genes		
				Phase I	Phase II	Phase III
CAR	Androgens, phenobarbital, rifampicin	DR-3, DR-4, DR-5, ER-6	Liver, intestine, kidney	CYP2A6, CYP2B1, CYP2B6, CYP2C9, CYP2C19	UGT1A1	ABCC2, ABCC3, ABCC4
SXR/PXR	Bile acids, steroids, rifampicin, phenobarbital, clotrimazole	DR-3, DR-4, DR-5, ER-6, ER-8	Liver, intestine	CYP1A2, CYP2B6, CYP2C9, CYP2C19, CYP3A4, CYP3A7, CYP7A1(-), CYP3A	SULT2A1, UGT1A1, UGT1A3, UGT1A4	ABCA1, ABCB1, ABCB11, ABCC1, ABCC2, ABCC3, ABCG2
FXR	Bile acids	IR-1, DR-1	Liver	CYP7A1(-), CYP8B1(-)	UGT2B4, SULT2A1	ABCB4, ABCB11, ABCC2
LXRα,β	Oxysterols	DR-4	Liver, intestine, kidney	CYP2B6(-), CYP3A4(-)		ABCA1, ABCG1, ABCG4, ABCG5, ABCG8
PPARα	Fatty acids, prostaglandins, leukotrienes, Fibric acids, phthalate esters	DR-1	Liver, intestine, kidney	CYP4A1, CYP4A3, CYP7A	UGT1A9, UGT2B4	ABCA1, ABCC2, ABCD2, ABCD3
PPARδ	Fatty acids, carboprostacyclin			CYP4A	UGT1A	ABCA1
PPARγ	Eicosanoids, thiazolidinediones			CYP4AB	UGT1A9	ABCA1, ABCG2
RXRα	Retinoic acids		Liver, intestine, kidney	CYP2B6		ABCB1, ABCG4
VDR	1α,25-dihydroxy vitamin D_3	DR-3, ER-6, IR-0	Liver, intestine	CYP2B6, CYP2C9, CYP3A4	SULT2A1	ABCC2

(-), down-regulation. (23)

Table 1.2. Nuclear receptor subfamilies

NR1 (Thyroid Hormone Receptor-like)
Group A (Thyroid hormone receptor): TRα/NR1A1; TRβ/NR1A2
Group B (Retinoid acid receptor): RARα/NR1B1; RARβ/NR1B2; RARγ/NR1B3
Group C (Peroxisome proliferators-activated receptor): PPARα/NR1C1; PPARβ/δ/NR1C2; PPARγ/NR1C3
Group D (Rev-ErbA): Rev-ErbAα/NR1D1; Rev-ErbAβ/NR1D2
Group F (RAR related orphan receptor): RORα/NR1F1; RORβ/NR1F2; RORγ/NR1F3
Group H (Liver X receptor-like): LXRα/NR1H3; LXRβ/NR1H2; FXR/NR1H4
Group I (Vitamin D receptor-like): VDR/NR1I1; PXR/NR1I2; CAR/NR1I3
NR2 (Retinoid X Receptor-like)
Group A (Hepatocyte nuclear factor-4): HNF-4α/NR2A1; HNF-4γ/NR2A2
Group B (Retinoid X receptor): RXRα/NR2B1; RXRβ/NR2B2; RXRγ/NR2B3
Group C (Testicular receptor): TR2/NR2C1; TR4/NR2C2
Group E: Human homologue of Drosophila tailless gene, TLX/NR2E1; Photoreceptor cell-specific nuclear receptor, PNR/NR2E3
Group F: Chicken ovalbumin upstream promoter transcription factor, COUP-TFI/NR2F1; COUP-TFII/NR2F2; V-erbA-related, EAR-2/NR2F6
NR3 (Estrogen Receptor-like)
Group A (Estrogen receptor): ERα/NR3A1; ERβ/NR3A2
Group B (Estrogen-related receptor): ERRα/NR3B1; ERRβ/NR3B2; ERRγ/NR3B3
Group C (3-Ketosteroid receptor): Glucocorticoid receptor, GR/NR3C1; Mineralocorticoid receptor, MR/NR3C2; Progesterone receptor, PR/NR3C3; Androgen receptor, AR/NR3C4
NR4 (Nerve Growth Factor IB-like)
Group A: Nerve growth factor IB, NGFIB/NR4A1; Nuclear receptor related 1, NURR1/NR4A2; Neuron derived orphan receptor 1, NOR1/NR4A3
NR5 (Steroidogenic Factor-like)
Group A: Steroidogenic factor 1, SF1/NR5A1; Liver receptor homolog 1, LRH-1/NR5A2
NR6 (Germ Cell Nuclear Factor-like)
Group A: Germ cell nuclear factor, GCNF/NR6A1
NR0 (Miscellaneous)
Group B: Dosage sensitive sex reversal, adrenal hypoplasia critical region on chromosome X, gene 1, DAX1/NR0B1; Small heterodimer partner, SHP/NR0B2
Group C: Nuclear receptors with two DNA binding domains, 2DBD-NR

(24)

Subfamily 1 is the biggest family with 7 groups. This subfamily is called the thyroid hormone receptor-like receptors, and includes TRs, RARs, PPARs, LXR, VDR, PXR, and CAR. Subfamily 2 contains the retinoid X receptor-like receptors, HNF4, RXRs, and testicular receptors. Subfamily 3 contains the estrogen receptor-like receptors, including estrogen receptors, estrogen-related receptors, and ketosteroid receptors. Subfamily 4 composes the nerve growth factor IB-like receptors, subfamily 5 contains steroidogenic factor-like receptors, subfamily 6 consists of germ cell nuclear factor-like receptors, and the last subfamily 0, is composed of miscellaneous receptors.

1.1.2 *Nuclear receptor structure*

Most nuclear receptors share a common modular structure which includes the N-terminal ligand independent transcription activation function domain AF-1, the DNA binding domain (DBD), a flexible hinge region, and the C-terminal ligand binding domain (LBD) which contains AF2 (Figure 1.1) (25).

The N-terminal AF1 domain is a structurally variable ligand-independent transactivation domain. Some studies showed that DNA-binding and protein-protein interactions can modulate AF1 conformation (26-28). The DBD of the nuclear receptors is composed of two highly conserved zinc fingers and is responsible for targeting a receptor-specific response element within regulatory regions of target genes (29,30). This helical globular domain is also responsible for dimerization (31). The function of the hinge region, which connects the DBD and LBD, is not clear. There is evidence that phosphorylation of this region increases transcriptional activation of some nuclear receptors (32,33).

The structure and function of the C-terminal LBD has been extensively investigated. This domain contains the ligand-binding site where specific xenobiotic or endogenous ligands bind and the conserved activation function-2 (AF-2) domain. Upon ligand binding, AF-2 recruits several coactivator proteins that facilitate chromatin remodeling and activation of the transcriptional machinery (14,34,35). The LBD is also critical for receptor dimerization and is required for higher affinity binding to response elements (36,37).

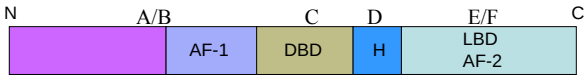


Figure 1.1. Nuclear receptor structural domains. AF, activation function; DBD, DNA binding domain; H, hinge region; and LBD, ligand binding domain.

1.1.3 *Nuclear receptor coregulators*

Most nuclear receptors, except those belonging to class I, regulate target genes based on exchange between corepressors and coactivators (coregulators are listed in Table 1.3 below) (13,14). In the absence of an agonist or in the presence of an antagonist, these nuclear receptors recruit corepressor complexes, such as nuclear receptor corepressor (NCoR) (38), silencing mediator of retinoic and thyroid hormone receptors (SMRT) (39), and Mi-2/NuRD complexes which exhibit both histone deacetylase and nucleosome dependent ATPase subunits that repress transcription (40,41). When nuclear receptors bind agonists, they undergo conformational changes,

resulting in the release of corepressor complexes and recruitment of coactivator complexes (36). More than 200 cofactors have been discovered and these include the p160 steroid receptor coactivator (SRC) family (35), histone modification enzymes (42), thyroid hormone receptor-associated proteins (TRAPs) (43), and other proteins. Coregulators with histone modification activity can also modify coactivators or even nuclear receptors and thereby regulate activation of the target genes. For example, p300 can acetylate the hinge region of the estrogen receptor alpha and this regulates transactivation and hormone sensitivity of this receptor (44).

Compared with corepressors, the structure and function of coactivators has been more extensively investigated. Coactivators play a role not only in histone modifications, chromatin remodeling and transcription initiation, but also in elongation, RNA splicing, and RNA maturation (45,46). Coactivators usually contain a LXXLL motif for binding with the nuclear receptor ligand binding domain (47). Histone modification enzymes are required to loosen the tight chromatin structure, and among coactivators, the SRC/p160 family and the cyclic AMP response element-binding protein (CBP)/p300 proteins exhibit histone acetyltransferase (HAT) activity (48). Protein arginine methyltransferase 4 (PRMT4/CARM1) and PRMT1 are both histone methyltransferases (HMTs) that are also nuclear receptor coactivators (49,50). ATP-dependent remodeling complexes such as SWI/SNF increase the chromatin accessibility (34,51); the positive transcription elongation factor (P-TEFb) plays a role in elongation (52); PGC-1 (peroxisome proliferator-activated receptor gamma coactivator-1), CAPER (coactivator of activating

protein-1 and estrogen receptors), and CoAA (an RRM-containing transcriptional coactivator) are important in mRNA splicing (45,53,54).

Table 1.3. Nuclear receptor coregulators.

COACTIVATORS
Acetyltransferases
CBP, p300, pCAF, GCN5, Tip60
Interact with CBP/p300
SRC-1/NCoA1, GRIP1/TIF2/SRC2/NCoA2, CIP/RAC3/AIB1/ACTR/TRAM1/SRC3
ATP-dependent chromatin remodeling complex
SWI/SNF/BRG1
Methyltransferases
CARM1, PRMT1,
TRAP/DRIP/ARC complex
COREPRESSORS
NCoR, SMRT, LCoR

(55)

It has also been reported that modification of nuclear receptors and other coregulators is important for regulating gene transcription and these modifications include acetylation, methylation, phosphorylation, ubiquitination, SUMOylation and other modifications (42,56-61). Modifications of coregulators can influence multiple gene sets targeted by different nuclear receptors that interact with the modified coregulator. Table 1.4 lists coregulator modifications and functions of these modifications (62).

Table 1.4. Nuclear receptor coregulator modifications.

Coregulator	Modification	Regulatory activity
C-terminal binding protein 1	Phosphorylation	Loss of repressor activity
N-CoR	Phosphorylation SUMOylation Ubiquitination	Nuclear export Increased repressive activity Increased protein turnover
PCBP1 (poly(rC) binding protein 1)	Phosphorylation	Loss of translational repression, conversion to a transcriptional coactivator, and control of alternative splicing
PGC-1a	Phosphorylation Methylation Acetylation	Increased transcriptional activity Decreased transcriptional activity Decreased transcriptional activity
RIP140 (receptor-interacting protein 140)	Vitamin B6 conjugation Phosphorylation Arginine methylation	Increased repressive activity Increased repressive activity Loss of repressive activity
SMRT (silencing mediator of retinoid and thyroid hormone receptors)	Phosphorylation	Nuclear export
SHARP (SMRT/HDAC1 Associated Repressor Protein)	Phosphorylation	Increased transcriptional repression
SRC-1	Phosphorylation	Increased transcriptional activity
SRC-2	Phosphorylation	Increased transcriptional activity
SRC-3	Phosphorylation Arginine methylation Acetylation Ubiquitination	Increased transcriptional activity, transcription factor-dependent transcription Transcriptional dynamics, protein dissociation Protein dissociation Increased transcriptional activity and protein turnover

(62)

In general, HAT containing coactivator complexes induce localized histone acetylation which is involved in gene activation. In addition to histone acetylation, coactivator SRC3 can by acetylated so that HAT-containing coactivator complexes dissociate and this results in decreased transactivation (63). Importin-α can be acetylated to regulate nuclear receptor translocalization (64); CBP/p300 can acetylate the nuclear receptor HNF-4 and prevent its transport from the nucleus to the cytoplasm (65). Other proteins, like p/CIP, AR and ERα can also be acetylated (66).

Coactivator associated arginine methyltransferase 1 (CARM1) binds to p160 proteins and synergistically activates target genes by methylating histone H3 (39). CBP/p300 can also be methylated by CARM1, and this modification suppresses the transcriptional activity of CBP/p300 (67) revealing the interplay between acetylation and methylation. Protein arginine methyltransferase 1 (PRMT1), another nuclear receptor coactivator that methylates histone H4 arginine 3 (H4R3) (50), can also methylate HNF-4 to increase DNA binding and enhance the transactivation (68). CBP/p300 and p160 proteins can be phosphorylated, and this also regulates their binding and interactions with other coregulators (69).

Kinase-mediated phosphorylation also modifies NRs and coregulators and influences then effects on target gene transcription. A signal-regulated transactivation domain was found in CBP/p300 to be controlled by cAMP, and nuclear calcium and calcium/calmodulin-dependent protein kinase IV (70). PGC-1alpha was reported to be phosphorylated by p38 MAPK, resulting in dissociation of the coactivator from p160 myb-binding protein (p160MBP), and loss of p160MBP-dependent repression (71).

The ubiquitin-proteosome system uses ubiquitin modifications to control protein levels. Poly-ubiquitinated proteins are subjected to degradation by proteosomes, while mono-ubiquitination is involved in transcriptional regulation rather than degradation, and mono-ubiquitinated proteins are stable (72). AR, PPARα, GR, RARγ, RXRα, and TRs are all regulated by the ubiquitin-proteosome system (73-77); and the nuclear receptor coregulator p300 has the ability to poly-ubiquitinate p53 protein (78).

The small ubiquitin-related modifier (SUMO) is an ubiquitin-like molecule that plays a role in the localization and stabilization of coregulators (79). For example, a SUMO conjugating enzyme, Ubc9, can interact with AR, and SUMO E3 ligases were found to repress AR transactivation (80). Sumoylation can activate and stabilize GR (81,82) and SRC-1, GRIP1, and PR are all regulated by sumoylation (83,84). Sumoylated Elk-1 recruits HDAC2 to deacetylate histones (85); HDAC6 is recruited by sumoylated p300 (86) and sumoylation by SUMO E3 ligase RanBP2 also modifies HDAC4 deacetylase activity (87).

1.2 PREGNANE X RECEPTOR (PXR)

1.2.1 *PXR expression and structure*

The pregane X receptor (PXR) was identified as NR1I2, a member of the NR1I nuclear receptor subfamily, and plays an important role in xenobiotic responses. It was first discovered in mouse and human for its activation by various natural and synthetic pregnanes and was named PXR, SXR (steroid and xenobiotic receptor), and PAR (pregnane activated receptor) by different research groups (88-90). Later, its orthologs were cloned in rat, rabbit, and dog (17,91,92).

The main tissues where PXR is expressed are the liver, small intestine, colon and kidney in human, rabbit, rat and mouse where CYP3A genes are induced (90,92-95). Other tissues also express low levels of PXR, such as lung, stomach, peripheral blood monocytes, blood-brain barrier, uterus, ovary, placenta, breast, osteoclasts, heart, adrenal gland, bone marrow and specific regions of the brain (93,96).

Compared to its ligand-dependent transactivation, the regulation of PXR expression is less clear. PXR expression can be regulated by other nuclear receptors. Pascussi et al. reported that micromolar concentrations of the GR agonist dexamethasone can increase PXR mRNA levels in humans and mice and enhance CYP3A gene expression and the GR antagonist RU486 can block this effect (97). Another research group demonstrated that PXR expression is positively regulated by HNF4α (98). Two PPARα ligands, clofibrate and perfluordecanoic induce PXR expression in rat hepatocytes (92), indicating a regulatory role of PPARα on PXR. Pregnant mice express about 50-fold higher PXR in the liver and ovary than normal mice. This suggests that PXR expression

might be stimulated by pregnancy-induced endogenous hormones and it functions to protect the fetus or mother from xenobiotics or other endogenous toxic chemicals (94). Interleukin-6 (IL-6) was demonstrated as an inhibitor of PXR expression during the acute phase response in infections (99). In addition, chromatin structure, phosphorylation-dependent protein degradation, and cellular trafficking can also control PXR gene expression level (100-102).

Subcellular localization of PXR is still controversial. Transfected human PXR localizes in the nucleus regardless of ligand treatment (103,104), and endogenous or injected mouse PXR in mouse liver was found localize in the cytoplasm and translocate into the nucleus upon ligand treatment (102,103). However, localization of the endogenous human PXR in primary hepatocytes has not been described.

The human PXR gene consists of 9 exons and spans approximately 35kb of genomic DNA on chromosome 3q12-13.3. Three splicing variants were found to express different PXR isoforms (93). Only a few polymorphisms of CYP3A4 were identified and have little effect on CYP3A4 expression or function (105) However, 70 SNPs (single nucleotide polymorphisms) of PXR have been investigated (105-110), providing a good candidate molecular mechanism for the >50-fold inter-individual differences in CYP3A4 induction in human. However, although functional differences have been demonstrated among SNPs, they are not sufficient to predict all individual differences in drug metabolism.

Although the overall domain arrangement of PXR is similar to other nuclear receptors, PXR can bind and is activated by a wide range of structurally diverse ligands.

The crystal structure of PXR LBD reveals several special features that distinguish it from other nuclear receptors. The volume of PXR ligand binding pocket is much bigger than other nuclear receptors. This is due to a 60-residue insertion unique to PXR which contains two additional β-strains (111-114). Crystal structure analysis demonstrated that PXR can expand highly mobile regions of its ligand binding pocket to fit large molecules such as rifampicin (111). Distinct from other nuclear receptors, the PXR LBD sequence is highly variable across species. This variability confers the diversity in response to different ligands (114) as discussed below. Some individual residues in the PXR LBD were critical for species-specific PXR transactivation (114-116).

1.2.2 *PXR ligands*

PXR can be activated by various pharmaceutical chemicals, environmental contaminants, dietary compounds, steroids, secondary bile acids, as well as several natural products. PXR LBD shares 70-80% identity across mammalian species, compared to 90% identity of other NR ligand binding domains and this is responsible for species-specific ligand activation profiles of PXR as well as species-specific CYP3A (cytochrome P450 3A) induction (114,117). CYP3A is a family of monooxygenases which exhibits phase I drug metabolizing enzyme activity (118). It was discovered that in different species, cytochrome P450 gene expression is induced differentially by known ligands (119) and these differences were, in part PXR-dependent. For example, rifampicin, an antibiotic that activates human and rabbit PXR and induces CYP3A4 expression, has little effect on mouse or rat PXR (17). In contrast, the antiglucocorticoid pregnenolone 16-a-carbonitrile (PCN), the major agonist for mouse and rat PXR

activates CYP3A11 expression, but does not activate PXR in humans or rabbits (17,120,121). Humanized mouse which expresses human PXR in mouse is a very useful animal model in studying human PXR ligands (122).

Human PXR can be activated by structurally diverse therapeutic chemicals. The PXR agonist first discovered are those that induce expression of cytochrome P450 3A (CYP3A) and include phenobarbital, metyrapone, clotrimazole, nifedipine, taxol, spironolactone, and trans-nonachlor (9,88,91,123-125). Other xenobiotics that activate PXR include ritonavir (the HIV protease inhibitor), tamoxifen, dexamethasone, 4-hydroxytamoxifen, the antidiabetic agent troglitazone, SR12813, bisphenol A, nonylphenol, and phthalic acid (17,120,126,127).

In addition to these xenobiotics, PXR can also be activated by a variety of endogenous ligands (9,90,128,129). All PXR orthologs were reported to be activated by the progesterone metabolite 5β-pregnane-3,20-dione (130). Other pregnanes that activate PXR include pregnenolone for mouse PXR, progesterone and its 17α-hydroxylated derivative for rabbit PXR, and estradiol for human PXR (9,17,90). These findings are consistent with high expression of PXR during pregnancy and suggests a role for PXR in protecting the fetus from endogenous toxic steroids (94). Toxic bile acids that activate PXR include lithocholic acid (LCA) and its 3-keto metabolite. (131). In addition to protecting the body from xenobiotic toxicity, PXR also protects body against harmful effects of toxic endobiotics (132,133).

The major natural products that activate PXR are St. John's wort and vitamin E. St. John's wort is an herb widely used for treatment of mild to moderate clinical depression

(134). Hyperforin, the major compound of St. John's wort was demonstrated to be a potent natural agonist for PXR. It induces PXR transcriptional activation and recruitment of the coactivator SRC-1 (135,136). These data explain the reported adverse interactions between St. John's wort and other prescription drugs (137). Among different forms of vitamin E, all four tocotrienols bind and activate PXR. However, tocopherols do not have this effect (138). In osteosarcoma cells, vitamin K2 activated PXR and other known PXR agonists induced the same bone markers as vitamin K2 (128). Other natural products that regulate PXR are reviewed by Staudinger et. al (139).

Several PXR antagonists have recently been discovered. The marine-derived drug ecteinascidin 743 (ET-743) blocks activation of human PXR by SR12813 or paclitaxel, and the induction of PXR target genes CYP3A4 and MDR1 (multidrug resistance protein 1) (140). Some highly chlorinated polychlorinated biphenyls (PCBs), were found to antagonize human PXR, although PCBs are potent rodent PXR activators (141). Ketoconazole disrupts binding of the corepressor SMRT and coactivator SRC-1 with PXR (142) and ketoconazole analogues fluconazole and enilconazole inhibit paclitaxel-induced PXR activation (143,144). Sulforaphane (SFN) is also a PXR antagonist (145).

1.2.3 *PXR target genes and functions*

Drug/xenobiotic metabolism consists primarily of three steps, known as phase I (oxidation), phase II (conjugation), and phase III (transportation) reactions. In phase I and II, the lipophilic xenobiotic molecules are converted into more hydrophilic metabolites so that they can be excreted from the body in phase III.

Phase I reactions include oxidation, reduction, hydrolysis, and some other reactions (146). Cytochrome P450 (CYP) are the major heme-dependent monooxygenases that catalyze phase I reactions (118). The CYP3A subfamily catalyzes oxidation of diverse xenobiotics in liver and intestine. Many CYP3A substrates also induce their expression (147) and evidence linking PXR to the regulation of CYP3A gene expression, includes the following observations: 1) PXR and CYP3A are both highly expressed in the liver and small intestine (92,95); and 2) almost all chemicals that induce CYP3A genes can activate PXR (88). The species-specific PXR agonists also induce species-specific CYP3A expression, as discussed above in 1.2.2 (94,125,148). 3) upon agonist activation, PXR forms a heterodimer with RXR and binds to xenobiotic response elements in CYP3A promoters (88-90,149). These elements contain two copies of consensus nuclear receptor binding motifs (AG(G/T)TCA), in the form of DR-3 (direct repeat of motif with a 3 bp spacer) in the CYP3A5, CYP3A7, CYP3A1, and CYP3A2 promoters and CYP3A4 enhancer (89,90). Other elements include an ER-6 (everted repeat with 6 bp spacer) in the CYP3A4 promoter (149), a DR-4 and ER8 in other target genes such as CYP2B, CYP2C, and MDR1, MRP2 (89,150-152). 4) The major PXR regulated CYP3A gene in mice, CYP3A11, was not induced by PCN or dexamethasone in the PXR-null mice. The PXR-null mice were sensitive to xenobiotics that are metabolized by CYP3A11 (122,131).

In phase I reactions, PXR also regulates other phase I monooxygenases, including CYP2B6, CYP2B9, CYP2C8, CYP2C9, and CYP2C19 (140,151,153,154). Other phase I drug metabolism genes regulated by PXR include carboxylesterases, dehydrogenases,

and some enzymes involved in heme production and the P450 reaction cycle (155,156). Phase II genes regulated by PXR include sulfotransferases, UDP-glucoronosyltransferases, glutathione-S-transferases, and carboxylesterases (157-162). PXR also regulates phase III drug efflux genes such as multidrug resistance 1 (MDR1), multidrug resistance associated protein 1, 2, 3 (MRP1A/B, MRP2, and MRP3), and breast cancer resistance protein (BCRP/ABCG2) (127,131,140,150,152,163). PXR not only plays an important role in drug metabolism but also regulates genes in bile acid synthesis, transport, and metabolism, including cholesterol 7α-hydroxylase and Na^+-dependent organic anion transporting polypeptide 2 (Oatp2) (131). Table 1.5 lists major PXR regulated genes in human and mouse.

1.2.4 *PXR coregulators*

Upon ligand binding, PXR forms a heterodimer with RXR and subsequently binds to response elements in target gene regulatory regions to activate gene transcription. Elaborate gene regulation is mediated by recruitment of coregulators and it has been suggested that binding of ligand changes the conformation of the AF-2 region in the C-terminal end of LBD and creates a coactivator-binding cleft (112).

Table 1.5. PXR regulated genes in human and mouse.

Gene	Function	Response elements
CYP3A4	Phase I metabolism	DR-3, DR-4, ER-6
CYP3A7		ER-6
mCYP3A11, mCYP3A13		
rCYP3A2		DR-3
rCYP3A23		DR-4
CYP2C9, CYP2C8, CYP2C19		DR-4
CYP1B1, CYP2B6, rCYP2B1/2, mCYP2B10		DR-4
CYP1A1, CYP1A2		
CYP2A6		
ALDH1A1, mALDH1A7, 1A1		
GSTA2, mGSTA1	Phase II metabolism	
UGT1A1, UGT1A3, UGT1A4, mUGT1a1		
SULT1A1, SULT2A1		
MDR1,	Phase III transporter	DR-4
mMRP1A/B,		
mMRP2,		ER-8
mMRP3, mOATP2		
rOATP2		DR-3
BCRP, BSEP, slc21a5		
ALAS1, mALAS1	Essential accessory proteins	
mPAPS synthase		
mPor		
cholesterol 7α-hydroxylase	Bile acid metabolism	
CYP7A1, CYP8B1	Bile synthesis	
CYP24	Vitamin D hydroxylation	

m, mouse; r, rat; the others, human. (164)

Several coregulators interact with PXR and the p160 family member SRC-1 (steroid receptor coactivator 1) was first PXR coactivator identified that binds PXR ligand-dependently, using the LXXLL repeats (90,165). Other coactivators that interact with PXR include glucocorticoid receptor-interacting protein 1 (GRIP1/SRC-2), SRC-3, peroxisome proliferator activated receptor binding protein (PBP), receptor interacting protein 140 (RIP140), peroxisome proliferators-activated receptor γ coactivator (PGC-1), and suppressor for Gal 1 (SUG-1) (94,100,120,129,166,167). Corepressors also interact with PXR and these include nuclear receptor corepressor (NCoR) (100,168). The interaction between silencing mediator of retinoid thyroid receptor (SMRT) and PXR is disrupted upon agonist treatment (169) and the small heterodimer partner (SHP) also interacts with PXR to repress its transcriptional activity (170).

Other proteins are recruited through the coactivators to enhance PXR-dependent transcription and these include the transcriptional coregulator p300. Like SRC-1, p300 exhibits histone acetyltransferase activity in remodeling chromatin and enhancing target gene transcription (171-174). SRC-1 also recruits coactivator-associated arginine methyltransferase 1 (CARM1) and synergistically enhances target gene transcription (49).

Interactions between PXR and coregulators can also be influenced by post-translational modifications. Phosphorylation of mouse PXR by protein kinase A enhances interactions between PXR and SRC1 or PBP (175). However, mouse PXR is phosphorylated by protein kinase C and this decreased interactions between PXR and SRC1 (100). RXRα phosphorylation at serine 32 also inhibits PXR activity (176).

1.2.5 *PXR interplays with other NRs*

CAR

The nuclear receptor not closely related to PXR is constitutive androstane receptor (CAR). These receptors functionally overlap in ligand binding and gene activation. Originally, it was shown that PXR regulates CYP3A and CAR regulates CYP2B genes (90,123,177,178). However, new studies showed that both CAR and PXR are activated by rifampicin and phenobarbital (130). PXR activates CYP2B genes through binding to the phenobarbital response element (PBPE), which is the CAR recognition site (122,151). CAR can also activate CYP3A genes through binding to DR-3 and ER-6 elements which are PXR/RXR binding sites (179-181). Other genes coregulated by PXR and CAR include CYP2C genes, glutathione-S-transferases, sulfotransferases, UDP-glucoronosyltrnasferases and MRP2 (152,153,182). CAR and PXR exhibit differences in specific genes they activate. For example, PXR regulates Aldh1a1, Aldh1a7, CYP3A11, GSTA1, GSTM2, and MDR1B which are not regulated by CAR. CAR regulates CYP1A1, CYP2A4, and SULTN which are not regulated by PXR (182).

FXR

Toxic secondary bile acids, such as lithocholic acid (LCA), are ligands for both PXR and FXR (129,131). LCA activates PXR and induces CYP3A and Oatp2, and represses CYP7A1 (183,184). Bile acids-dependent activation of FXR induces small heterodimer partner SHP an inhibitor of liver receptor homolog-1 (LRH-1), which is responsible for induction of CYP7A1 (185). SHP can inhibit PXR and PXR can also regulate SHP (170,186). FXR also regulates CYP3A4 through binding to the response

elements in the CYP3A4 promoter (123). FXR binding elements also exist in the PXR promoter and PXR is directly regulated by FXR (187).

VDR

Vitamin D plays a role in the maintenance of bone density by activating VDR and activation of VDR target gene CYP24 regulates vitamin D levels (188-190). Many PXR agonists can cause osteomalacia. CYP3A4 was recently shown to metabolize vitamin D in human liver and small intestine, whereas CYP24 expression is very low (190). Further evidence showed that PXR inhibits VDR-mediated CYP24 expression (191). VDR also activates CYP3A4 upon ligand treatment and SULT2A1 is another gene regulated by both PXR and VDR, and by CAR (192).

1.3 CHROMATIN AND GENE REGULATION

As described above, nuclear receptors (NRs) are ligand-dependent, DNA-binding transcription factors. In eukaryotic organisms, DNA is compacted in chromatin, in the nucleus and the chromatin remodeling is very critical for the transcriptional machinery to access target gene promoters. Many enzymes that remodel nucleosomes or modify histones are nuclear receptor coactivators.

1.3.1 *Chromatin structure*

DNA was considered as the storage facility for all genetic information; however, after completion of the human genome project, there were several unsolved mysteries that cannot be simply explained by DNA information. For example, the number of protein coding genes in human is 35,000 only, twice the number of *Drosophila* genes. It

is also known that the genetic profiles of human and mouse share 99% homology. In addition, the whole organism is developed from a single zygote. How does it divide and differentiate into so many cell types? Different cells express different genes and develop different morphology and functions yet all somatic cells share identical genome sequences.

Epigenetics is an emerging field of science that is beginning to provide some answers to these questions. In their review, David Allis and Emily Bernstein described epigenetics broadly as "a phenomenon that changes the final outcome of a locus or chromosome without changing the underlying DNA sequence", and more specifically as "the study of any potentially stable and, ideally, heritable change in gene expression or cellular phenotype that occurs without changes in Watson-Crick base-pairing of DNA" (193). DNA and histone modifications can influence the chromatin structure and thus influence gene expression, which provides an important molecular mechanism for epigenetic phenomena.

In eukaryotic cells, DNA is packaged by histones and nonhistone chromosomal proteins (such as HMG proteins) into a condensed structure called chromatin. By this packaging, a 2-meter-long strand of DNA is compacted in the tiny nucleus with a diameter of around five micrometers. The primary package structure is the basic unit called the nucleosome, as shown in Fig. 1.2, with a 147 base pair core DNA wrapping an octamer of core histones for two rounds. The octamer core histone is composed of a heterotetramer of histones H3 and H4, and two heterodimers, H2A and H2B. The nucleosomes are connected by a 10-60 bp linker DNA bound with linker histone H1 and

a nonhistone HMG protein (194-197). In a higher order, nucleosomes are packaged into 30nm-wide chromatin and further condensed into 100 nm-wide chromosomes during mitosis. This highly packaged chromatin structure represents an obstacle for gene transcription by restricting transcription factor binding (195). To activate genes, DNA must be released from the highly compacted nucleosome structure to allow recruitment of RNA polymerase II transcriptional apparatus to the regulatory regions and exert transcription initiation and elongation (198,199). In general, there are two processes that can execute this function. ATP-dependent chromatin remodeling by SWI/SNF or NURF can change regional chromatin structure and expose the DNA to facilitate activation (200,201). The other process is histone modification.

1.3.2 *Histone modifications*

The C-terminal major domains of core histones form globular structures and interact with each other (196,202). The highly dynamic N-terminal tail of each histone protein extends from the surface and interacts with DNA, other nucleosomes, or cofactors (203). A large number of amino acids in this domain are dynamically modified and play an important role in gene regulation, signal transduction, chromosome modeling, DNA repair, DNA replication, and other processes (204-206). Mistakes in histone modifications may be important in human diseases, such as cancer (207).

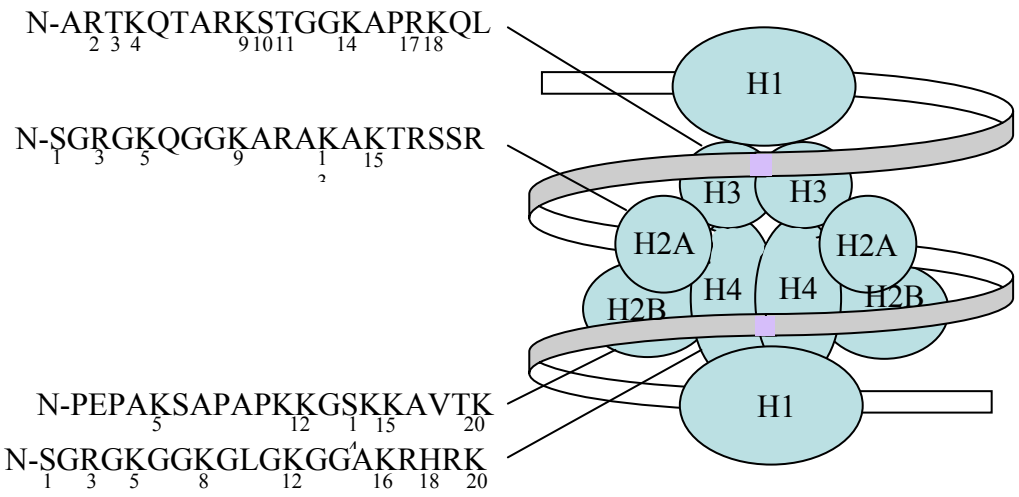


Figure 1.2. Structure of nucleosome. 147 bp core DNA wraps a core histone octamer for two rounds. 10-60 bp linker DNA binds with linker histone H1. The amino-terminal tails of each histone protrude, containing specific sites for post-translational modifications, which are marked by their sequential numbers.

The histone modifications include phosphorylation (208), acetylation (209), methylation (210), ubiquitylation (211), sumoylation (212), ADP ribosylation (213), deimination (214,215), and proline isomerization (216). Those modifications on histones affect the chromatin structure or provide a binding site for coregulators (205). Based on what type of modification takes place and which amino acid is modified, distinct cellular processes are associated. Also, several different enzymes are responsible for modifications on specific amino acids. A list of histone modification enzymes is presented in Table 1. 6.

1.3.2.1 Histone acetylation

Histone acetylation has been correlated with active transcription *in vivo* for over 35 years (217). Histone acetylation homeostasis is controlled by two types of enzymes, histone acetyltransferases (HATs) and histone deacetylases (HDACs) (218-220). Approximate 30 known HATs are divided to two types, type A-HATs which acetylate histone to regulate chromatin remodeling and gene transcription, and type B-HATs which acetylate histone in the cytoplasm before histone incorporation into newly replicated chromatin (221-223). There are 18 mammalian HDACs, divided to three classes. Class I HDACs include HDAC-1, -2, -3, -8, and -11. Class II include HDAC-4, -5, -6, -7, -9, and -10. Class III HDACs are SIRT (silent mating type information regulation 2 homolog) proteins (224).

1.3.2.2 Histone methylation

Histone methylation takes place on lysine and arginine. Lysines 4, 9, 27, and 36 of histone H3 and lysine 20 of H4 are targets for lysine methyltransferases. These lysine methyltransferases share a conserved 130 amino acid SET-domain (205). Arginine methyltransferases are discussed in detail later. H3K79 is a residue in the core domain that can be methylated in yeast for gene silencing (225,226). Histone methylation has long been considered irreversible. However, recent studies provided evidence of histone demethylase activity with several proteins, including LSD1, and JMJD2 subfamily members (227-229).

Table 1.6. Histone modification enzymes

Enzymes	Residues modified
Acetyltransferase	
HAT1	H4 (K5, K12)
CBP/P300	H3 (K14, K18) H4 (K5, K8) H2A (K5) H2B (K12, K15)
PCAF/GCN5	H3 (K9, K14, K18)
TIP60	H4 (K5, K8, K12, K16) H3K14
HB01(ScESA1, SpMST1)	H4 (K5, K8, K12)
ScSAS3	H3 (K14, K23)
ScSAS2(SpMST2)	H4K16
ScRTT109	H3K56
Deacetylase	
SirT2(ScSir2)	H4K16
Lysine methyltransferase	
SUV39H1, SUV39H2	H3K9
G9a	H3K9
ESET/SETDB1	H3K9
EuHMTase/GLP	H3K9
CLL8	H3K9
SpClr4	H3K9
MLL1-5	H3K4
SET1A, SET1B	H3K4
ASH1	H3K4
Sc/Sp SET1	H3K4
SET2 (Sc/Sp SET2)	H3K36
NSD1	H3K36
SYMD2	H3K36
DOT1	H3K79
Sc/Sp DOT1	H4K20

Enzymes	Residues modified
Lysine methyltransferase	
Pr-SET 7/8	H4K20
SUV420H1-2	H4K20
SpSet9	H4K20
EZH2	H3K27
RIZ1	H3K9
Lysine Demethylase	
LSD1/BHC110	H3K4
JHDM1a	H3K36
JHDM1b	H3K36
JHDM2a	H3K9
JHDM2b	H3K9
JMJD2A/JHDM3A	H3K9, H3K36
JMJD2B	H3K9
JMJD2C/GASC1	H3K9, H3K36
JMJD2D	H3K9
Arginine Methyltransferase	
CARM1	H3 (R2, R17, R26)
PRMT1	H4R3, H2A R3
PRMT5	H4R3, H3R8
Serine/Thrionine Kinase	
Haspin	H3T3
MSK1, MSK2	H3S28
CKII	H4S1
Mst1	H2BS14
Ubiquitylase	
Bim1/Ring1A	H2AK119
RNF20/RNF40	H2BK120
Proline isomerase	
ScFPR4	H3P30, H3P38

(230)

Interplay between different modifications is complex and provide mechanisms for the functions of histone modifications. A number of proteins that recognize specific modification and also exhibit enzymatic activity have been identified. BPTF recognizes H3K4me3 and recruits the NURF chromatin-remodeling complex to activate downstream gene (231). JMJD2A and CHD1 proteins bind to H3K4me mark to perform histone lysine demethylase and ATPase activities, respectively (232-234). Polycomb protein PC2 recognizes H3K27me and recruits ubiquitin ligase for H2A. Different modifications cannot occur on the same lysine. Recent evidence has also shown that one site-specific modification can influence adjacent modifications. For example, phosphorylation of H3S10 reduces binding of HP1 to H3K9me (235). Isomerization of H3P38 also reduces methylation of H3K36 by Set2 (216). H3S10 phosphorylation facilitates GCN5 acetyltransferase recognition of H3 (236). Ubiquitinylation of H2B is required for methylation of H3K4me3 (237,238).

1.3.3 *Histone modifications and gene transcription*

Histone modifications function to establish a global chromatin environment, euchromatin or heterochromatin, and to facilitate DNA-based functions which include transcription, DNA repair, and DNA replication.

Silent heterochromatin and active euchromatin exhibit different histone modifications. Heterochromatin, which is important during separation of chromosomes, has low level of total acetylation and high level of methylation on H3K9, H3K27, and H4K20. H3K9 methylation is recognized by heterochromatin associated protein HP1 and

is further stabilized by HP1 to maintain the heterochromatin state (239). Hyperphosphorylation is also linked to condensed chromatin (220).

In a euchromatin environment, a transcription factor is recruited to the promoter upon stimulation, and promotes modifications which result in gene activation or repression. Histone modifications in the promoter or on the coding region may exhibit opposite functions (240).

1.3.3.1 Acetylation

Acetylation is primarily associated with gene activation. Acetyltransferases, such as CBP/p300, GCN5, have been identified as coactivators (48,55). Most HATs modify multiple lysines on the N-terminal tail of histones (48). Only lysine 56 on H3 is a lysine within the core domain that can be acetylated (241-243). The hypothesis that histone acetylation changes the surface charges and destabilize the histone-DNA interaction is supported by observation that acetylated histones are easier to displace from DNA (244-246). Another mechanism is that histone modifications including acetylation can be recognized by other proteins and result in gene activation or repression, depending on those proteins that recognize the modification. In contrast, histone deacetylases (HDACs) correlate with gene expression. Most HDACs don't have much preference for specific sites. However, H4K16 acetylation is a special case that it is deacetylated by yeast sir2 and its human analogues (247). Different from other acetylation, the function of H4K16 acetylation is to inhibit the formation of 30nm chromatin fibers (248).

1.3.3.2 Methylation

Histone methylation catalyzed by histone methyltransferases (HMTs), which contain a conserved SET domain (205) can occur on lysine or arginine (249,250). Activation associated H3K4 methylation is mediated by the SET1 complex in yeast and it distributes across the whole ORF (211). The MLL/WRD5 complex recognizes dimethylated H3K4 and generates trimethylation on this residue in humans (251). In yeast, H3K4me3 primarily localizes to the 5' end and is associated with RNA Pol II (252). Several proteins with chromating remodeling or histone modification activities also contain domains that recognize this methylation, including chromatin-remodeling factor NURF and Chd1, mouse SIN3/HDAC, and human Tip60. H3K36 methylation mediated by Set2 is also associated with elongation. Both di- and trimethylation of this lysine mainly localize to 3' ORF and associates with elongation form of Pol II. EAF3 protein recognizes this methylation and brings the Rpd3S deacetylase complex to the ORF. Acetylation in this region is removed to prevent inappropriate internal transcription (253-255).

Methylation on H3K9 in the promoter facilitates binding of the heterochromatin protein 1 (HP1) to chromatin. HP1 recruits other factors including SUV39H1 which methylate H3K9 and HDACs leading to heterochromatic gene silencing (249,256). HP1 family members also interact with DNM1 and trigger DNM1 to methylate DNA, which is another repression marker (257). Methylation on H3K27 and H4K20 has been implicated in heterochromatin formation.

Histone arginine methylation can be associated with gene activation or repression. Two members of protein arginine methyltransferases (PRMTs), PRMT1 and PRMT4/CARM1, were discovered as nuclear receptor coactivators (50,63). CARM1 specifically methylates arginines 2, 17, and 26 of H3, while PRMT1 specifically methylates arginine 3 of H4 (258). In the estrogen-regulated pS2 promoter, arginine methylation was observed to be cyclic during activation (259). Proteins that recognize or remove arginine methylation have not been identified. Deimination by PADI4 converts arginine to citrulline and prevent arginine methylation (214,215). Mono- but not dimethylated arginine can also be converted to citrulline (215). The reverse process, converting citrilline to arginine has not been reported. JMJD6, a member of the lysine demethylase family, was reported to demethylate H3R2 and H4R3 (260).

1.3.3.3 Phosphorylation

Histone phosphorylation is involved in chromosome condensation, gene activation, response to DNA damage, and induction of apoptosis (261). Phosphorylation of serine 10 H3 (H3S10) occurs during mitosis and alters gene regulation (262). Phosphorylation of H3 by ribosomal S6 kinase 2 (RSK-2) and MAP and stress-activated kinase 1 (MSK-1) is responsible for this phosphorylation and intracellular signal transduction (263,264). Phosphorylation of linker histone H1 by cyclin-dependent kinase 2 (cdk2) causes its dissociation from chromatin and influence on gene transcription (265).

These different histone modifications interact with each other. The active or repressive transcriptional stage may be dictated by a series of modifications. For example, CARM1 and p300 show synergism in estrogen receptor-mediated gene

regulation (49). Methylation of H4R3 by PRMT1 can increase acetylation of H4K8 and H4K12 by p300, although preacetylation of H4 will reduce the H4R3 methylation by PRMT1 (50). SET9 methylation of H3 stimulates the subsequent acetylation of H3 and H4 by p300, while SUV39H1 methylation of H3 inhibits the same acetylation (266).

1.4 PROTEIN ARGINING METHYLTRANSFERASES (PRMTs)

1.4.1 *Protein arginine methyltransferase overview*

Protein arginine methyltransferases (PRMTs) are members of the methyltransferase family that transfer a methyl group from S-adenosylmethionine (SAM or AdoMet) to the guanidine group of protein arginine residues (267). PRMTs have been discovered in many species, including fungi, plants, invertebrates, and vertebrate animals (267-270). Nine mammalian PRMTs have been identified, PRMT1-9, while only one was observed in yeast, Hmt1/Rmt1 (271). PRMTs are classified as type I or type II. PRMT1, -3, -4, -6, and -8 are type I enzymes that form monomethylarginine (MMA) or asymmetric dimethylarginine (ADMA). PRMT5 is a type II PRMT, which produces MMA and symmetric dimethylarginine (SDMA) (271,272). The other three, PRMT2, PRMT7, and PRMT9 were discovered by homology and their activities have not yet been determined (273).

Proteins with glycine and arginine rich (GAR) motifs are known substrates of PRMTs. PRMT1, -3, and -6 usually recognize the GAR motif. PRMT1 was shown to recognize Arg-Gly-Gly repeats (274). CARM1/PRMT4 does not recognize the GAR motif but methylates other specific substrates (275). PRMT5 and PRMT7 recognize and

methylate isolated arginines or arginines in GAR motifs (276). A list of known PRMT substrates is given in Table 1.6.

Table 1.7. Partial listing of histone and non-histone substrates for PRMTs

PRMT	Type	Histone substrate	Nonhistone substrate
PRMT1	Type I	Histone H4, H2A	Fibrillarin, Nucleolin, Sam68, FGF-2, STAT1, STAT3, EWS, SAF-A, NIP45, Mre11, hnRNP A1, hnRNP A2, hnRNP R, hnRNP K, SPT5, CIRP, ILF3, TLS/FUS, RNA helicase A, TAFII68, RBP58, ZF5, p137GP1, SAMT1
PRMT3	Type I		rpS2, PABPN1
PRMT4/ CARM1	Type I	Histone H3	PABP1, p300/CBP, HuR, TARPP, Sm B/B’
PRMT5	Type II	Histone H4, H2A, H3	MBP, Sm D1, Sm D3, Sm B/B’, coilin, LSm4, SPT5
PRMT6	Type I		PRMT6, Fibrillarin, HIV tat
PRMT7	Type II		Fibrillarin

(271)

Methylation of an arginine residue can alter the interaction between the modified protein and other proteins or molecules, resulting in altered functions of the methylated proteins (205). The outcome of protein methylation by PRMTs can result in alteration of transcriptional regulation, signal transduction pathways, nuclear-cytoplasmic transport, and posttranscriptional modification of gene expression (271).

1.4.2 *PRMTs function as coactivators*

Several PRMTs are coactivators of nuclear receptors. They can methylate receptors, coregulators, transcriptional factors, and histones to regulate transcription. CARM1

methylates the N-terminus of histone H3 and was the first PRMT identified as a coactivator that is recruited through p160 coactivators to nuclear receptors bound to promoter regions of specific genes (277). PRMT1, that methylates histone H4 and was also identified as a nuclear receptor coactivator that binds to p160 (278). The associations with p160 are required for both CARM1 and PRMT1 coactivator functions (44,49,279). Methylation of H3R17 and H3R26 by CARM1 and methylation of H4R3 by PRMT1 are also transcriptionally active markers (50,205). The methyltransferase activity of PRMT2 has not been described and this methyltransferase is also a coactivator for AR and ERα (280) and inhibits IκB-α export from nucleus and thus inhibit NF-κB transcription (281). PRMTs are also identified as coactivators of p53, YY1, PPARγ, RUNX1, and E2F1 (282,283).

It has also been suggested that arginine methylation interacts with other protein modifications, thereby facilitating transcriptional activation. Synergistic cooperations between CARM1 and PRMT1 (279), and between CARM1, CBP/p300 and pCAF have been reported (284). PRMT1, CARM1 and p300/CBP were recruited through p160 (279). PRMT1 methylation of histone H4 was detected to increase the subsequent acetylation of H4 by p300 (50) and acetylation of nucleosomes in p53-dependent transcription (285). An *in vivo* study showed that PRMT1 methylation of H4 is essential for subsequent histone modifications (286). Preacetylated histone H4 by p300 is inhibitory to methylation by PRMT1 (50); however, preacetylated H3 by p300 enhances methylation by CARM1 (287).

Besides interplay between histone modifications, CARM1 also interacts with ATP-dependent chromatin remodeling enzymes. CARM1 forms a complex with SWI/SNF factors and functions in many ways (288). First, the interaction between CARM1 and Brg1 increases Brg1 ATPase activity and changes CARM1 preference for free histone H3 to nucleasomal H3 (67). Second, a nuclear receptor coactivator, protein Flightless I, binds CARM1, Brg1, and the other SWI/SNF component BAF53 (289), which form a connection between histone modification and ATP-dependent chromatin remodeling.

PRMTs also methylate nonhistone proteins to activate transcription. CBP/p300 are activators for many transcription factors, including nuclear receptors, cAMP response element binding protein (CREB), signal transducer and activator of transcription 1 (STAT1), and activator protein-1 (290). Mutations of arginine residues reduced the coactivator function of CBP (49,284), indicating that methylation may be required for its coactivator functions. STAT1 methylation by PRMT1 at arginine 31 prevents binding of the PLAS1 inhibitor and promotes its activation (291). Receptor interacting protein 140 (RIP140), a nuclear receptor corepressor, is methylated by PRMT1 to inhibit its repressive activity (292). Some small nuclear ribonucleoprotein particles (snRNPs, like SmD1 and SmD3), some heterogeneous nuclear ribonucleoproteins (hnRNPs), HNF4, a transcriptional factor, and HuR, an mRNA-stabilizing protein must be methylated for full function (68,275,293).

1.4.3 *PRMTs function as corepressors*

PRMT5 is the first PRMT identified as a corepressor for several transcription factors, including E2F1, Brg1, hBRM, Blimp1, and Snail (294-297). PRMT5 was known

to cause symmetrical dimethylation of H3R8 and H4R3 (298). However, its repression mechanism is not clear. PRMT6 is responsible for H3R2 methylation, which prevents MLL1 and WDR5 from methylating H3K4. In this way, it repress transcription by blocking the active H3K4 methylation mark (299-301). PRMT7 was described critical for DNA methylation of the imprint control region (302).

PRMT1 and CARM1 also play a role in repression; for example, SPT5 is a transcriptional elongation factor that can be methylated by PRMT1 and PRMT5 to abolish its binding with RNA pol II, and therefore inhibit its elongation activity (303). Although CARM1 performs synergistic activation with CBP/p300; CARM1 methylation of CBP/p300 prevents its interaction with transcription factors, resulting in inhibition of CBP/p300 coactivator functions (67,304).

The high mobility group protein (HMG) is a family of proteins which binds to linker DNA between nucleosomes to stabilize the chromatin structure (305). AT hook is a motif in HMG proteins that can be methylated. It has been suggested that methylation of AT hooks may be related to protein-DNA or protein-protein interactions (306).

1.4.4 *Regulation of PRMTs*

The enzyme activities of PRMTs can be regulated by their binding proteins, BTG1 and BTG2 (307). A BTG1-binding protein, hCAF1 exhibits substrate-dependent regulation of PRMT1 (308). DAL-1 can inhibit PRMT3 activity (309). Within NUMAC (nucleosomal methylation activator complex), CARM1 can methylate nucleosomal H3, while it only methylates free H3 *in vitro* (288). Binding to BRG and BRM enhances PRMT5 activity, and binding to CTCFL enhances PRMT7 activity (298,302).

Modification of PRMTs can also regulate their activity. CARM1 phosphorylation prevents its homodimerization and inhibit its enzyme activity (310). Automethylation of PRMT1, -4, -6, and -8 were reported without clear functional consequences (311).

Other modification marks usually prevent the adjacent arginine to be methylated. H3K9 acetylation inhibits H3R8 methylation (298). H3R8 methylation also inhibits H3K9 methylation (312). H3K4 trimethylation but not mono- or dimethylaiton can inhibit H3R2 methylation by PRMT6 (299,301). H3R2 also inhibits H3K4 methylation (300).

CHAPTER II

EPIGENETIC REGULATION OF TRANSCRIPTIONAL ACTIVITY OF PREGNANE X RECEPTOR BY PROTEIN ARGININE METHYLTRANSFERASE 1

2.1 OVERVIEW

Pregnane X receptor (PXR) is an orphan nuclear receptor that regulates metabolism and disposition of various xenobiotics and endobiotics (313). These physiological functions of PXR are achieved through coordinating transcriptional regulation of Phase I and Phase II drug-metabolizing enzymes as well as the "Phase III" transporters (164). The structural flexibility in the ligand binding pocket enables PXR to function as a xenobiotic receptor through interacting with a wide range of structurally diverse compounds (114). Xeno- and endobiotics that activate PXR include a variety of prescription and nonprescription drugs, herbal medicines, environmental toxicants, and bile acids (314).

Post-translational modifications on the N-termini of histones have been shown to play critical roles in gene regulation including the regulation of transcriptional activity by nuclear receptors. These modifications include phosphorylation, acetylation, methylation, and ubiquitination (230). It is believed that combination of

---

*This research was originally published in Journal of Biological Chemistry. Ying, Xie. Epigenetic Regulation of Activity of Transcriptional PregnaneX Receptor by Protein Ariginine Methyltransferase 1. *Journal of Biological Chemistry*. 2009; 284: 9199–9205. Copyright the American Society for Biochemistry and Molecular Biology.

modifications of the chromatin-associated histone and non-histone proteins, and the interplay between these modifications create a marking system ("histone code"), which is part of the epigenetic mechanisms for gene regulation (205).

The protein arginine methyltransferases (PRMTs) including PRMT1, PRMT2, and PRMT4 (CARM1) were shown to be nuclear receptor coactivators (277,279,315,316). These enzymes regulate gene expression through methylating histone and non-histone proteins, and the methylation marks are important for the nuclear/steroid hormone receptor-mediated transcriptional activity. PRMT1 is a major arginine methyltransferase which methylates arginine 3 of histone H4 and H2A. Recent evidence suggests that histone modification by PRMT1 sets the stage for subsequent histone modifications (286), and there is an intricate interplay between H4R3 methylation and other histone modifications. For example, arginine methylation (H4R3) by PRMT1 facilitates H4 acetylation but H4 acetylation inhibits methylation of H4R3 (50). These observations suggest that histone modifications during transcription proceed in a unidirectional sequence and in order to complete a transcription cycle, methylated H4R3 has to be demethylated, followed by acetylation and then deacetylation or replacement (317). Understanding the role of these histone modification enzymes in nuclear receptor-regulated gene expression will help us understand the epigenetic mechanism of gene regulation and provide important basis for drug/therapeutic designs to effectively intervene in pathological processes such as tumorigenesis and inflammatory responses.

In this study, we identified PRMT1 to be a major HMT associated with PXR, and we demonstrate that PRMT1 is a required histone methyltransferase for PXR

transcriptional activity. PRMT1 regulates PXR transcriptional activity by direct association with PXR in a ligand-dependent manner, and the PXR agonist rifampicin caused recruitment of PRMT1 to the regulatory region of PXR target gene *CYP3A4*. Knockdown of PRMT1 through siRNA or gene deletion inhibited PXR-dependent transcriptional activity. Furthermore, we found that PXR plays a critical role in regulating PRMT1 cellular compartmentalization and substrate preference.

2.2 MATERIALS AND METHODS

2.2.1 *Materials*

DMSO, rifampicin, PCN, anti-FLAG M2 antibody, and anti-FLAG M2-agarose affinity beads were from Sigma. Core histones were from Roche (Indianapolis, IN). *S*-Adenosyl-L-[*methyl*-3H]-methionine ([3H]SAM) and [35S]-methionine were from PerkinElmer (Waltham, MA). Recombinant histone H4, acetylated H4 N-terminal peptides, recombinant PRMT1, as well as anti-acetyl-(pan) H4, anti-H4 (Me2) R3, and anti-PRMT1 antibodies were purchased from Upstate (Millipore, Billerica, MA). Goat and mouse anti-PXR antibodies, and isotype IgGs were from Santa Cruz Biotechnology (Santa Cruz, CA). Nitrocellulose and polyvinylidene difluoride membranes were from Bio-Rad.

2.2.2 *Cells*

HepG2 and CV-1 cells were cultured in Dulbecco's modified Eagle's medium (HyClone, Logan, UT) supplemented with 10% fetal bovine serum (Sigma) and 1x antibiotic and antimycotic (Invitrogen, Carlsbad, CA). PXR-HepG2 and PXR-HT29

stable transfectants were created as described in (318). Wild-type and PRMT1-null ES cells were obtained from Mark Bedford (MD Anderson, Houston, TX) and cultured according to (319).

2.2.3 *Plasmids*

Plasmids expressing GST-fused PXR fragments have been created in our laboratory. DNA sequences coding different PXR fragments were PCR-amplified and subcloned into pGEX-5X-3 expression vector (Amersham Biosciences). pACT, pBIND, and pG5-luc were purchased from Promega (Madison, WI) for the mammalian two-hybrid assay. pBIND-PXR (Gal4-PXR) and pACT-PRMT1 (VP16-PRMT1) were constructed by inserting PCR-amplified human PXR DNA sequence into pBIND vector and PCR-amplified PRMT1 DNA sequence into pACT vector following the manufacturer's recommendation (Promega).

2.2.4 *Co-immunoprecipitation (co-IP)*

PXR-HepG2 cells were washed with PBS and homogenized in the Co-IP lysis buffer (20 mM Hepes, pH 7.4, 125 mM NaCl, 1% Triton X-100, 10 mM EGTA, 2 mM Na3VO4, 50 mM NaF, 20 mM ZnCl2, 10 mM sodium pyrophosphate, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride). Complete protease inhibitor mixture (Sigma) was added before use. Mouse liver tissues were homogenized in the same (above) lysis buffer. After centrifugation (12,000 x g in a microcentrifuge at 4 °C for 15 min), supernatant fractions were collected and incubated with antibodies and GammaBind Plus-Sepharose beads (Amersham Biosciences) for 2 h at 4 °C on a rotary shaker. Corresponding isotype IgG was used as a negative control. The beads were

washed three times, and the precipitated protein complexes were analyzed with HMT assay or Western blot.

2.2.5 *Western Blot*

Proteins were analyzed by SDS-PAGE and then transferred to a nitrocellulose membrane. After over 4 h of blocking in 5% milk with TBST buffer (20 mM Tris-HCL, pH 7.6, 137mM NaCl, 0.5% Tween 20), the blot was incubated with appropriate primary antibodies at 37 °C overnight. After washing with TBST buffer for 30 min, the membrane was then subjected to 1:2000 corresponding alkaline phosphatase-conjugated secondary antibodies for 2 h. After another wash with TBST for 30 min, the membrane was exposed to Nitro Blue tetrazolium/BCIP as the substrate (Promega).

2.2.6 *Histone Methyltransferase Assay*

The PRMT1 (Upstate) HMT assay was based on the manufacturer's recommendation. In brief, 2 μg of core histones, 2 μg of H4, or 0.4 μg of H4 N-terminal peptides were incubated with the immunoprecipitated HMT complexes or recombinant PRMT1 at 30 °C for 90 min, in 1xHMT buffer (50 mM Tris, pH 9.0, 1 mM phenylmethylsulfonyl fluoride, 0.5 mM dithiothreitol) with *S*-adenosyl-L-[*methyl*-3H]methionine ([3H]SAM) as the methyl donor. The reaction mix was separated in 16% SDS-PAGE, and the separated proteins were transferred to the polyvinylidene difluoride membrane for autoradiography and staining with Ponceau BS red dye (Sigma).

2.2.7 *Peptide Sequencing Analysis*

The radioactive proteins identified by autoradiography were excised from the membrane for N-terminal sequencing by Edman degradation. The radioactivity in the

Edman degradation fractions corresponding to amino acid residues was determined by liquid scintillation counting.

2.2.8 *GST pull-down assay*

The GST pull-down assay was performed as described (52). Briefly, [35S]methionine-labeled fulllength PRMT1 protein was generated with a TNT-coupled Reticulocyte Lysate System (Promega) using the SP6 promoter-driven cDNA plasmid as the template. PCR-generated PXR cDNA fragments were inserted in-frame into pGEX-5X-3 (Amersham Biosciences). The plasmids were expressed in *Escherichia coli* (BL21), and fusion polypeptides were purified with glutathione-Sepharose 4B beads (Amersham Biosciences) according to the manufacturer's instruction. Twenty micrograms of each fusion polypeptide (estimated by comparison with bovine serum albumin in an SDS-PAGE gel with Coomassie Blue staining) was incubated with 20 μl of radiolabeled PRMT1 in a total volume of 200 μl of binding reaction buffer (20 mM Hepes pH 7.9, 1% Triton X-100, 20 Mm dithiothreitol, 0.5% bovine serum albumin, and 100 mM KCl) for 3 h at 4 °C. After incubation, beads were washed three times with the same buffer without bovine serum albumin. The bound proteins were eluted by boiling in the SDS-PAGE sample buffer and resolved by 12% SDS-PAGE gel electrophoresis. The signals were detected by autoradiography. The input control was 2 μl of the radioactive PRMT1.

2.2.9 *Transient transfection and Luciferase assay*

Cells were seeded in 12-well plates. When growth reached 50% confluence, cells were transfected with plasmid DNA for 12 h using Lipofectamine (Invitrogen). The

transfected cells were treated with chemicals or vehicle for an additional 48 h. The luciferase assay was performed using a luciferase assay system kit, according to the manufacturer's recommendation (Promega).

2.2.10 *Mammalian two hybrid assay*

The mammalian two-hybrid assay was performed using Checkmate Mammalian Two Hybrid System (Promega). CV-1 cells were seeded in 12-well plates and transient transfected with pBIND-PXR, pACT-PRMT1, and pG5-luc as shown in table 2.1. 12 h after transfection, cells were treated with rifampicin (10 μM, 48 h), and luciferase activity was determined with Polarstar optima luminometer (BMG Laboratory).

Table 2.1. Experimental design for the mammalian two hybrid assays.

Transfection	pBIND	pACT	pG5-luc
1	pGal4-PXR	pACT	pG5-luc
2	pGal4-PXR	pACT-PRMT1 full length	pG5-luc
3	pGal4-PXR	pACT-PRMT1 SET domain	pG5-luc
4	pGal4-PXR	pACT-PRMT1 N-terminus	pG5-luc
5	pGal4-PXR	pACT-PRMT1 C-terminus	pG5-luc

According to the Checkmate Mammalian Two Hybrid System manual from Promega

2.2.11 *Small interference RNA*

Two small interfering RNA-expressing plasmids were constructed by cloning the sequences targeting PRMT1 at coding region sequences 756–773 (308) (siPRMT1–28) and 353–371(siPRMT1–11) into pSilencer 5.1 plasmids according to the manual (Ambion). The targeting plasmids were created by inserting 5'-

GATCCGATCCACTGGTGGGAGAACTTCAAGAGAGTTCTCCCACCAGTGGATT TTTTTGGAAAAGCT-3' (siPRMT1–28), and 5'-GATCCGCTCCATGTTTCATAACCGGTTCAAGAGACCGGTTATGAAACATGGA GTTTTTTGGAAAAGCT-3' (siPRMT1–11). The siRNA plasmids and the scramble siRNA control were co-transfected with PXR-directed reporter plasmid pGL3-*3A4*-Luc (318) into PXR-HepG2 cells. The transfected cells were treated with rifampicin (10 μM, 48 h). Luciferase activity and PRMT1 protein expression were determined with luminometry and Western blotting, respectively.

2.2.12 *Chromatin Immunoprecipitation (ChIP)*

ChIP assay was performed according to the manufacturer's protocol from Upstate, using the ChIP assay kit with modifications. Briefly, PXR-HepG2 cells were treated with rifampicin (10 μM, 2 h) and DMSO (vehicle control). Cells were cross-linked with 1% formaldehyde for 15 min at room temperature, and then the reaction was stopped by incubating in glycine with a final concentration of 0.125 M for 5 min. Cells were washed three times with cold PBS and harvested by scraping with cell scraper. Then the cells were lysed in the SDS lysis buffer (1% SDS, 10 mMEDTA, and 50 mM Tris-HCl, pH 8.1) on ice for 10 min. The samples were sonicated into DNA fragments of 0.2–1 kb (checked by agarose gel electrophoresis/ethidium bromide staining) and microcentrifuged at maximal speed for 10 min at 4 °C. The supernatant was precleared by rotating with 60 μl of salmon sperm DNA/protein-agarose slurry for 30 min at 4°C and then aliquoted after centrifugation. 20 μl was saved as input and 200 μl (equal to one-fifth the amount of cells from one 100% confluent 15-cm dish) was used for each

antibody. Each 200-μl supernatant was diluted with 800 μl of ChIP dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl, pH 8.1, and 167 mM NaCl) and incubated with the specific antibody (1 μg/sample) at 4°C overnight. A mock precipitation without antibody was used as negative control. The next day, 60 μl of salmon sperm DNA/protein-agarose slurry was added to each sample and incubated at 4°C for another 2–4 h. The beads were then washed for 3–5 min with 1 ml of each buffers listed: low salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 150 mM NaCl), high salt wash buffer (0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris-HCl, pH 8.1, 500 mM NaCl), and LiCl wash buffer (0.25 M LiCl, 1% IGEPAL-CA630, 1% deoxycholic acid (sodium salt), 1 mM EDTA, 10 mM Tris-HCl, pH 8.1). After all washes, pellets were suspended by vertex with 150 μl of freshly prepared elution buffer (0.1 M NaHCO3, 1% SDS) for 15 min, and then supernatant was collected. This elution progress was repeated once again, and in total 300 μl elutes were collected. The one-tenth input was diluted with dilution buffer to a total volume of 300 μl. Elutes and diluted inputs were incubated in 0.3 M NaCl at 65°C for 4 h to reverse formaldehyde cross-linking. Then 10 μl of 0.5 M EDTA, 20 μl of 1 M Tris-HCl, pH 6.5, and 20 μg of proteinase K were added to the sample and incubated at 45°C for 1 h. DNA was extracted with phenol/chloroform and then incubated with 10 μg of glycogen in 75% ethanol at -20°C overnight. After precipitation by centrifuging at 12,000 x *g* for 30 min at 4°C, the recovered DNA pellets were dissolved in 30 μl of distilled water. The DNA target in the sample was determined by realtime quantitative PCR in triplicates with a 1-μl sample. Three independent experiments were performed.

Amplifications were performed in the 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA) with SYBR Green Master Mix (Applied Biosystems). The PCR primers used were: forward primer, 5'-GTCCCAATTAAAGGTCATAAAGC-3' and reverse primer, 5'-CTTGAACCGACATGATTTCAAG-3'.

2.2.13 ***Statistical analysis***

Statistical evaluations were conducted using two-tailed *t* test with triplicates for each treatment. A *p* value of less than 0.01 was considered to be statistically significant. Data are the means $\pm$ S.D. of three independent results.

2.2.14 ***Immunofluorescence microscopy***

Cells were seeded in chamber slides and kept in standard cell culture conditions. For microscopy, the cells were washed with PBS and then fixed with freshly prepared 4% paraformaldehyde in PBS at 4°C for 10 min. After three washes, cells were permeabilized with 0.2% Triton X-100 in PBS for 10 min at room temperature. After another three washes, cells were blocked with 10% donkey serum in PBS/Tween (0.1% Tween20 in PBS) at room temperature for 3 h. Primary antibodies (mouse anti-PXR antibody with a dilution of 1:100, rabbit anti-PRMT1 antibody with a dilution of 1:500) in the blocking buffer were incubated with cells at 4°C overnight. The corresponding isotype IgG was used as negative control. After washing with PBS/Tween for three times, cells were incubated with anti-mouse red-fluorescent Alexa Fluor 568 or anti-rabbit green-fluorescent Alexa Fluor 488 dyes (Invitrogen) in the PBS/Tween for another 2 h at room temperature. Cells were washed for three times and DAPI (Vector

Laboratory, Burlingame, CA) was added. The results were analyzed by fluorescence microscopy (Olympus IX71) equipped with Olympus DP70 digital camera.

2.3 RESULTS

2.3.1 *Association of PRMT1 with PXR in HepG2 cells*

Histone methyltransferases are transcriptional regulators of nuclear receptors. To analyze PXR-associated histone methyltransferase(s) (HMT), we created a cell line (PXR-HepG2), by stable transfection of FLAG-tagged human PXR into HepG2 cells, which lack PXR (318). The HMT(s) associated with rifampicin-activated PXR were detected by co-immunoprecipitation followed by histone methyltransferase assay with core histones as the substrates and radiolabeled *S*-adenosyl-L-[*methyl*-3H]methionine ([H3]SAM) as the methyl donor. Methylated histones were detected by autoradiography following SDS-PAGE. As shown in Fig. 2.1A, the methyltransferase activity was associated with FLAG-tagged PXR in the precipitated complex and the precipitated HMT(s) methylated both histones H4 and H2A.

To identify the amino acid residue(s) methylated by PXRassociated HMT(s), the methylated H4 was analyzed by N-terminal sequencing. As shown in Fig. 2.1B, arginine

3 (H4R3) was the only methylated residue among the 23 N-terminal amino acids analyzed. PRMT1 is the predominant enzyme responsible for this site-specific methylation (320). H2A shares the same N-terminal "SGRGK" sequence motif (Fig. 2.1C) with H4, therefore it was also methylated by PRMT1 (Fig. 2.1A, left lane). PRMT5 is another enzyme that methylates H4R3 in this SGRGK motif (321). However, PRMT5 is also known to methylate histone H3 (298). Under our experimental conditions, we found that major HMT associated with PXR-methylated H2A and H4, but not H3.

It has been reported that acetylation of H4 inhibits methylation of H4R3 by recombinant PRMT1 (50). The pre-acetylation on H4K5, K8, K12, or K16 inhibits the methylation at similar level (50). However, in our experiments, the pre-acetylation on K12 significantly inhibited methylation of H4R3 by the PXR associated complex while pre-acetylation on the other lysines was less effective in the inhibition (Fig. 2.1D). The recombinant PRMT1 showed the same substrate methylation preference regardless the acetylation status of the test peptide. These results suggested that the substrate specificity of PRMT1 can be regulated when it is in association with PXR.

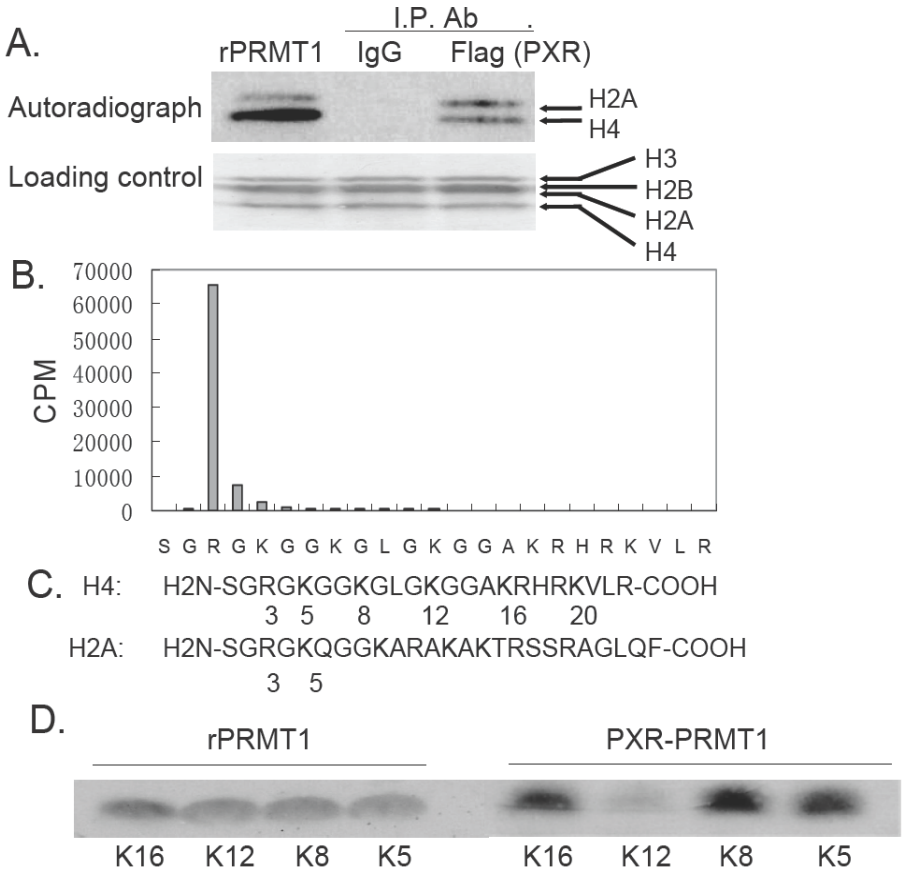


Fig. 2.1. Histone Histone methyltransferase activity is associated with PXR. A, PXR-HepG2 cells were treated with rifampicin (10 μM, 2 h) and used to perform co-immunoprecipitation/HMT assay with anti-FLAG antibody. The precipitates and recombinant PRMT1 were subjected to HMT assay, respectively, with core histones as the substrates and [3H]SAM as the methyl donor. Methylated histones were analyzed by autoradiography. B, methylated H4 in *A* was subjected to N-terminal sequencing analysis. The radioactivity associated with Edman degradation fractions was determined by liquid scintillation counting. C, illustration of N-terminal sequences of histone H2A and H4 with the common "SGRGK" motif. D, substrate specificity comparison of the PXRassocated HMT and recombinant PRMT1. Same molar pre-acetylated H4 N-terminal peptides (0.4 μg, 2 kDa) and recombinant H4 (2 μg, 11 kDa) (Upstate) were used as the substrates and [3H]SAM was used as the methyl donor. The pre-acetylated H4 peptides are 20-amino acid N-terminal peptides with K5, K8, K12, or K16 individually acetylated. Methylation was detected by autoradiography.

2.3.2 *Ligand-dependent physical and functional interaction between PRMT1 and PXR*

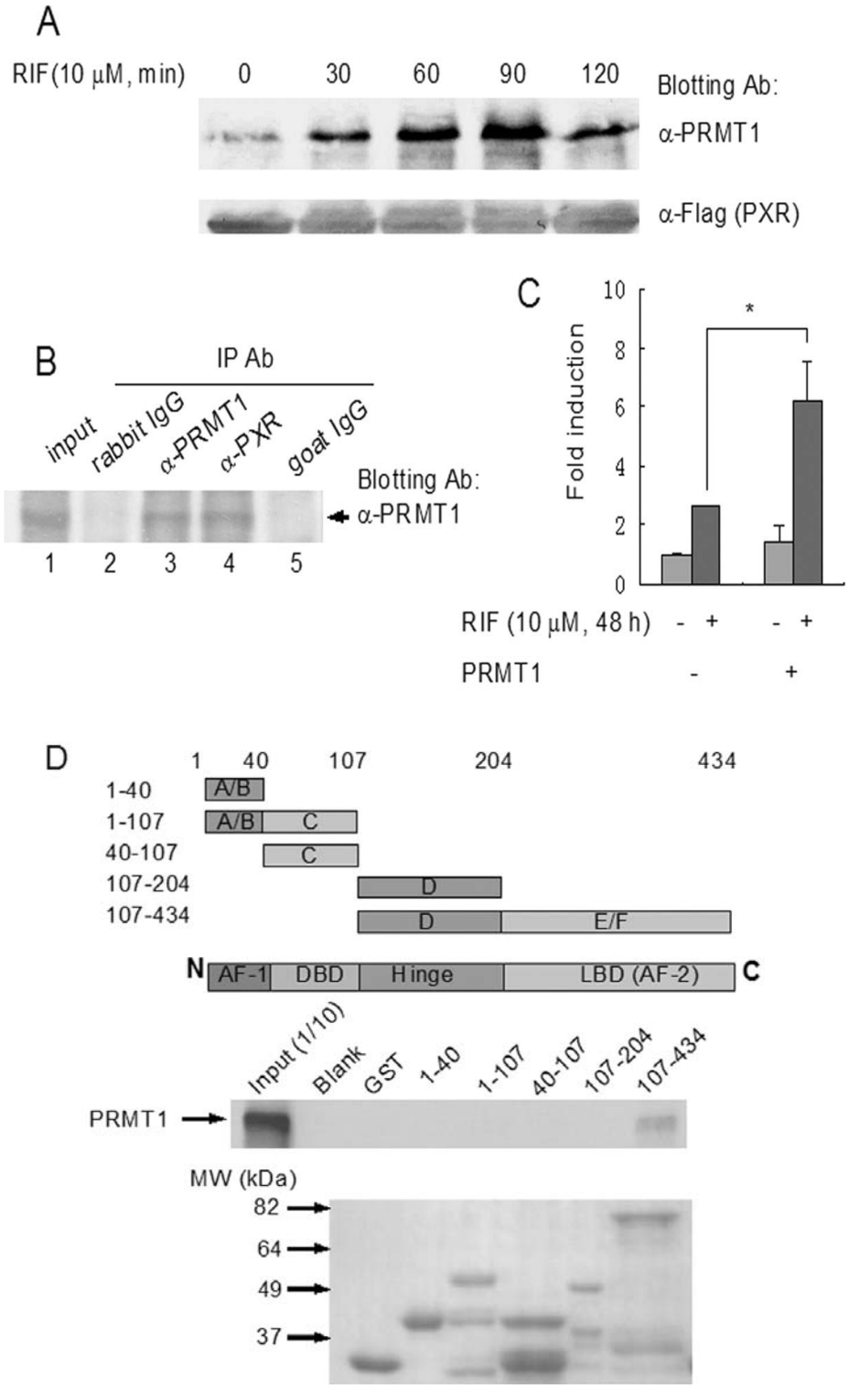
To analyze the effects of PXR ligand on the interaction between the receptor and PRMT1, we performed co-immunoprecipitation assay with the protein extracts of PXR-HepG2 cells treated with the PXR ligand rifampicin. The complexes precipitated with anti-FLAG antibody were eluted with FLAG tag and analyzed by Western blotting. PRMT1 was found to associate with PXR in a ligand-dependent manner (Fig. 2.2A).

To further analyze interactions between PXR and PRMT1 *in vivo*, we performed co-immunoprecipitation assay with liver tissues from VP16-hPXR transgenic mice. In these mice, the mouse PXR has been replaced with human PXR, which has been fused with VP16 activation domain, resulting in constitutively active PXR in these animals (122). PRMT1 was found to specifically associate with PXR as determined by co-immunoprecipitation followed by Western blot analysis (Fig. 2.2B).

To further analyze the ligand-dependent PXR-PRMT1 interaction, we performed mammalian two-hybrid assay in CV-1 cells. Consistent with the ligand-dependent interaction in the co-immunoprecipitation assay (Fig. 2.1A), transient transfection of VP16-PRMT1 significantly enhanced the Gal4-PXR-driven luciferase expression upon PXR ligand rifampicin treatment (Fig. 2.2C).

To identify and characterize the interactive domains of PXR responsible for association with PRMT1, we performed GST pull-down assay using GST fusion peptides containing various domains of PXR (Fig. 2.2D). As shown in Fig. 2.2D, only the PXR fragment, which contains the hinge domain and the ligand binding domain, interacted with PRMT1. However, the hinge domain alone showed no interaction. Taken

Fig. 2.2. PRMT1 interacts with PXR in a ligand-dependent manner. A, PXR-HepG2 cells were treated with rifampicin (10 μM, 0, 30, 60, 90, 120 min) and subjected to co-immunoprecipitation with anti-FLAG antibodycoupled beads. The precipitates were eluted with 3x FLAG peptide and analyzed by Western blotting with PRMT1 antibody. Anti-FLAG antibody blotting was used to show the equal loading of the samples. B, liver tissue from a VP16-hPXR transgenic mouse was homogenized in the Co-IP lysis buffer and co-immunoprecipitated with goat anti-PXR (lane 4) and rabbit anti-PRMT1 antibodies (lane 3). Goat IgG (lane 5) and rabbit IgG (lane 2) were used as negative controls. 1:10 lysate was loaded as the input control (lane 1). Precipitates were analyzed by Western blotting with PRMT1 antibody. C, CV-1 cells were transfected with the bait plasmid, pBIND-PXR, and the reporter pG5-luc vector, with cotransfection of the prey plasmid pACT-PRMT1 or blank pACT plasmid. Six hours after transfection, cells were treated with rifampicin (10 μM) or vehicle for an additional 48 h. The interaction was characterized by luciferase activity. *, statistically significant difference (*t* test, $p < 0.01$). The data are the means $\pm$ S.D. of three independent results. D, mapping of the interactive domains of PXR with PRMT1 by GST pull-down assay. Various PXR fragments were fused with GST and the fusion peptides coupled with glutathione-Sepharose beads were incubated with radiolabeled PRMT1. The precipitated complexes were analyzed by autoradiography following SDS-PAGE (middle panel). Upper panel, illustration of PXR fragments. Lower panel, loading control of the GST-fused PXR fragments (Coomassie Blue staining).


A
RIF(10 μM, min)
0
30
60
90
120
Blotting Ab:
α-PRMT1
α-Flag (PXR)
B
IP Ab
input
rabbit IgG
α-PRMT1
α-PXR
goat IgG
Blotting Ab:
α-PRMT1
1
2
3
4
5
C
Fold induction
10
8
6
4
2
0
*
RIF (10 μM, 48 h)
- + - +
PRMT1
- +
D
1
40
107
204
434
1-40
1-107
40-107
107-204
107-434
A/B
C
D
E/F
N
AF-1
DBD
Hinge
LBD (AF-2)
C
Input (1/10)
Blank
GST
1-40
1-107
40-107
107-204
107-434
PRMT1
MW (kDa)
82
64
49
37

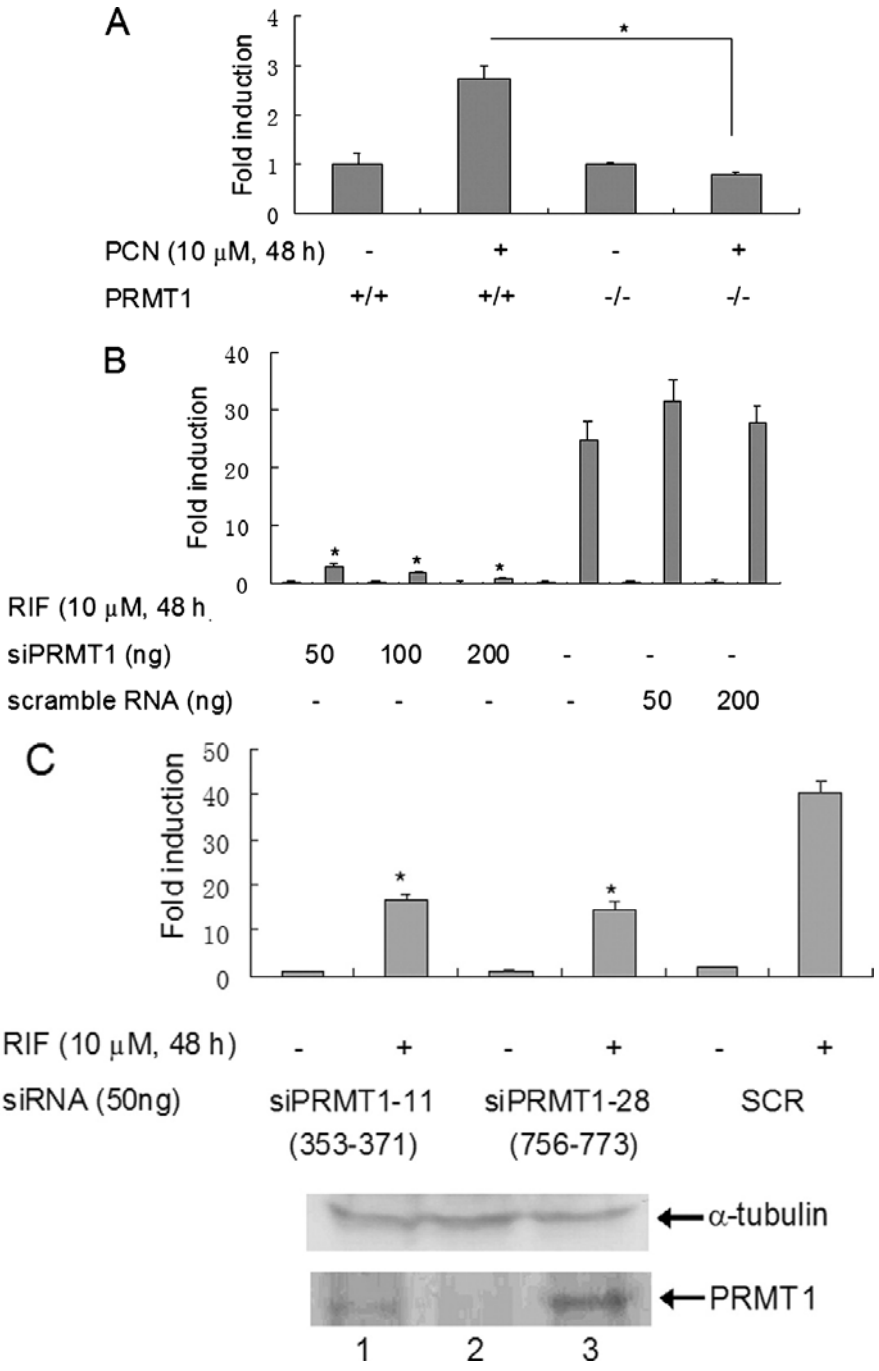
together, these results indicated that PRMT1 specifically associated with PXR ligand-binding domain, which is consistent with ligand-dependent interactions. The PXR interaction appears to be PRMT1-specific. PRMT5 failed to interact with GST-PXR fusion peptides in the pull-down assay (data not shown).

2.3.3 *Requirement of PRMT1 for the Transcriptional Activity of PXR*

The PRMT1-null mutation is embryonic lethal in homozygous mice. However, mouse embryonic stem cells survived without PRMT1 (322). We utilized these PRMT1 knock out mouse embryonic stem cells (ES) to analyze the role of PRMT1 in regulating PXR transcriptional activity *in vivo*. Gal4-mPXR (mouse PXR) and Gal4-responsive tk-UAS-luciferase reporter plasmids were co-transfected into the PRMT1-deficient and wild-type mouse ES cells. In the wild-type ES cells, mouse PXR agonist pregnenolone-16-α-carbonitrile (PCN) induced the PXR-driven luciferase reporter gene, whereas in the PRMT1 (-/-) ES cells, PCN was not effective in the induction (Fig. 2.3A).

To test the effect of PRMT1 on PXR transactivation in human cells, we performed PRMT1 knockdown experiment in PXR-HepG2 cells with a small interfering RNA (siRNA). The DNA fragment, which encoded a 21-bp hairpin siRNA targeting at PRMT1 nucleotides 756 –773 (308) was cloned into the pSilencer vector (Ambion). When this siRNA-expressing plasmid was transfected into the PXR-HepG2 cells, the PXR ligand-dependent activation of *CYP3A4*-luciferase reporter gene activity was dramatically inhibited (Fig. 2.3B). A similar result was obtained with another siRNA, which targets at PRMT1 nucleotides 353–371 (Fig. 2.3C, upper panel). The decreased

Fig. 2.3. PRMT1 is required for PXR transcriptional activity. A, PXR activity in PRMT1 (-/-) ES cells. Gal4-driven luciferase reporter gene and Gal4-mPXR were transiently transfected into mouse PRMT1-null ES cells or wildtype ES cells. The transfected cells were treated with the receptor agonist PCN (10 μM, 24 h). Luciferase activity was determined by a luminometer. *, statistically significant difference (*t* test, $p<0.01$). The data are the means ± S.D. of three independent results. B and C, the effect of siRNA knockdown of PRMT1 on PXR transcriptional activity. PXR-HepG2 cells were transfected with *CYP3A4*-luciferase. Two siRNAs targeting different sequences of PRMT1 (756–773 and 353–371) were used to knockdown PRMT1. Scrambled siRNA was used as the control (B and upper panel of C). The total PRMT1 protein expression was analyzed by Western blotting with PRMT1 antibody. Western blot with α-tubulin antibody was shown for loading control (C, lower panel). Lane 1, siPRMT1–11; lane 2, siPRMT1–28; lane 3, control. The reporter gene expression was measured by luciferase assay. *, statistically significant difference (*t* test, $p<0.01$). The data are the means ± S.D. of three independent results.


A
Fold induction
PCN (10 μM, 48 h)
PRMT1
+/+
-/-
B
Fold induction
RIF (10 μM, 48 h)
siPRMT1 (ng)
scramble RNA (ng)
C
Fold induction
RIF (10 μM, 48 h)
siRNA (50ng)
siPRMT1-11
(353-371)
siPRMT1-28
(756-773)
SCR
α-tubulin
PRMT1
1
2
3

PRMT1 protein expression in siRNA-transfected cells was confirmed by Western blotting analysis (Fig. 2.3C, lower panel).

2.3.4 *Recruitment of PRMT1 to the Regulatory Regions of PXR Target Gene CYP3A4*

PXR was identified as the major transcription factor that regulates *CYP3A4* through binding to the xenobiotic response enhancer module (XREM) which is about 8-kb upstream of the transcription starting site (123). The above results of physical and functional interactions between PXR and PRMT1 led us to hypothesize that PRMT1 is recruited to this regulatory region of *CYP3A4* in response to PXR ligand stimulation. PXR-HepG2 cells were used in a ChIP assay to analyze the recruitment of PXR and PRMT1 as well as changes of histone modifications on the *CYP3A4* regulatory region. Our results indicated that activation of PXR by rifampicin resulted in recruitment of PXR to the regulatory region of *CYP3A4* as well as increasing of histone H4 acetylation which is indicative of transcriptional activation of the gene. Concomitantly, PRMT1 was also recruited to this *CYP3A4* regulatory region in response to rifampicin treatment with increases in H4R3 methylation (Fig. 2.4).

2.3.5 *Regulation of PRMT1 Subcellular Localization by PXR*

Results from the co-immunoprecipitation/HMT assay indicated that PXR regulates PRMT1 substrate specificity (Fig. 2.1D). Because PXR and PRMT1 interacted physically and functionally, it is possible that PXR may influence the cellular distribution of PRMT1. To test this possibility, we examined the subcellular localization of PRMT1 in cells with or without PXR. Interestingly, in human hepatoma cell line HepG2 which lacks PXR, PRMT1 was primarily localized in the cytoplasm region.

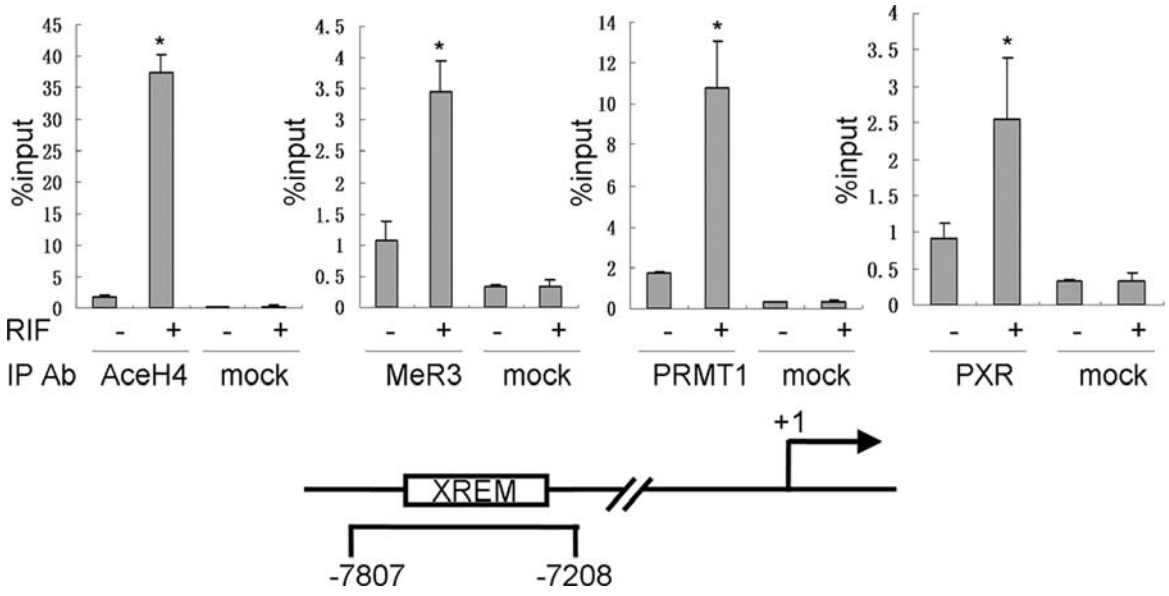


Fig. 2.4. Recruitments of PRMT1, PXR, and changes of histone modifications in the *CYP3A4* regulatory regions in response to PXR activation. PXR-HepG2 cells were treated with rifampicin (10 μM, 2 h). ChIP assay was performed to analyze the association of PXR, PRMT1, and changes of histone H4 acetylation and H4R3 methylation. Results were analyzed by quantitative real-time PCR. *, statistically significant difference (*t* test, $p < 0.01$). The data are the means ± S.D. of three independent results.

However, in PXR-HepG2 cells where PXR is restored, PRMT1 was largely localized in the nucleus. Similar effects of PXR on PRMT1 localization were also observed in the human intestinal epithelial tumor cell line HT29, which also lacks PXR. In these cells, PRMT1 was mainly localized in the cytoplasm. Upon restoration of PXR expression by stable transfection, PRMT1 became localized in the nucleus (Fig. 2.5). These results suggest that PXR plays an important role in regulating the nuclear compartmentalization of PRMT1, which may affect the activity of PRMT1.

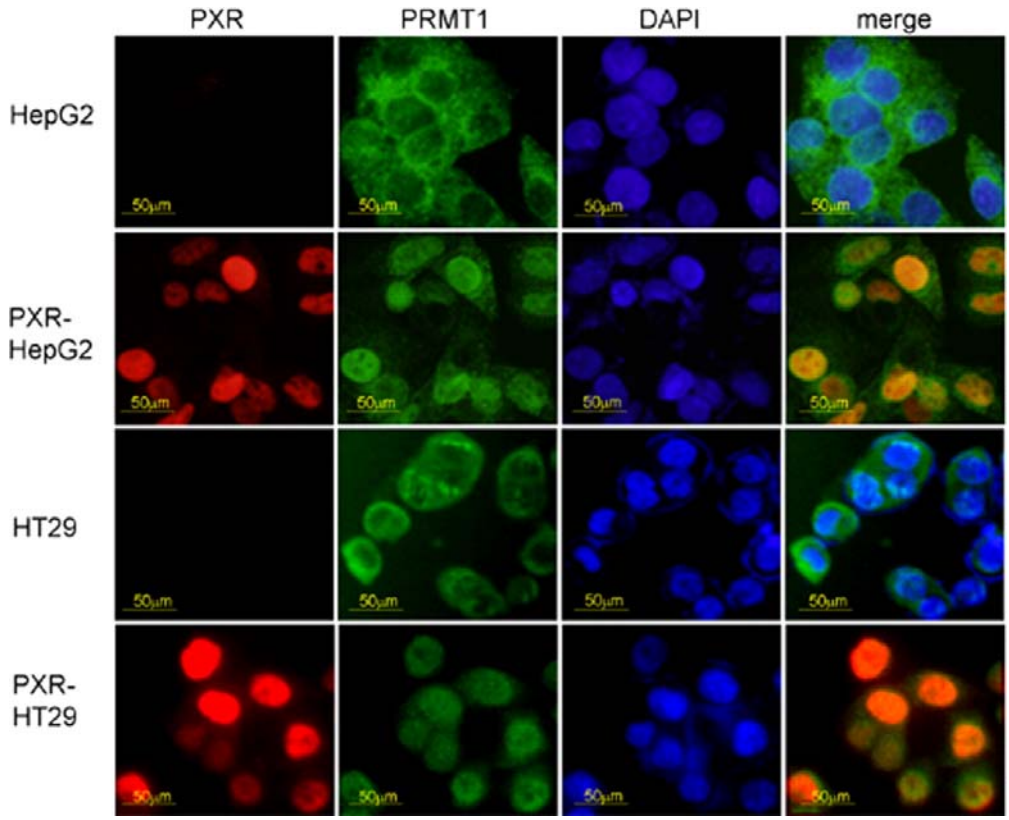


Fig. 2.5. PXR regulates PRMT1 subcellular localization as determined by immunofluorescence microscopy. Parental HepG2 and HT29 cells as well as the PXR stable transfectants PXR-HepG2 and PXR-HT29 were analyzed for PXR and PRMT1 subcellular localization by immunofluorescence microscopy.

2.4 SUMMARY AND DISCUSSION

PRMT1, which methylates arginine 3 of histone H4 (H4R3), is a major arginine methyltransferase in mammalian cells. Accumulating evidence indicates that PRMT1 plays a vital role in physiological and pathophysiological processes including development, nuclear receptor regulated gene expression, and oncogenesis (12, 25–27).

The mouse homozygous null mutant of PRMT1 is early embryonic lethal, attesting to the vital function of PRMT1 in the development and survival of the whole organism (23). In addition to modifying histones, PRMT1 methylates non-histone proteins involved in DNA repair (28), DNA methylation (29), translational control, and maintenance of heterochromatic and euchromatic barrier (30), suggesting that PRMT1 regulates many aspects of gene expression. At the molecular level, intricate interplay between PRMT1 and other histone modification enzymes have been observed. For example, methylation of H4R3 by PRMT1 promotes acetylation of histone H4, which leads to gene activation; however, acetylation of histone H4 inhibits H4R3 methylation (14), suggesting a unidirectional relay of histone marking processes in a transcription cycle (15). Based on our results, we propose that by direct interaction with PRMT1, nuclear receptors such as PXR initiate target gene transcription by recruiting PRMT1 to the regulatory region to accomplish the step of creating methyl marks on the chromatin.

In this study, we provide strong evidence indicating that PRMT1 is a major histone methyltransferase associated with PXR and plays an indispensable role in the transcriptional activity of PXR. We used an unbiased biochemical approach with FLAG-tagged PXR to precipitate the PXR-associated histone methyltransferases in HepG2 cells. In our Co-IP/methyltransferase assay with core histones H3, H2A, H2B, and H4 as substrates, H2A and H4 were methylated by the PXR-associated HMTs. We sequenced the N-terminal 23 amino acids of methylated H4 and found that H4R3 was the major methylated residue. The methylated H2A was most likely due to the common “SGRGK” motif shared by these peptides (Fig. 2.1C).

The results of Co-IP in both mouse liver tissue and PXR transfected HepG2 cells indicated that PXR interacts with PRMT1. GST pull-down assay strongly suggested that the direct interaction between PXR and PRMT1 is through the PXR ligand binding domain. As indicated by the ChIP assay results, this direct interaction may play a role in recruitment of PRMT1 to the *CYP3A4* regulatory region, where it promotes transcription through methylation and acetylation of chromatin as demonstrated in Fig. 2.4. Another possibility is that by direct contact, PRMT1 methylates PXR and thus modifies its transactivation. For example, HNF-4α is methylated by PRMT1 and thus changes its activity in gene regulation (25). We have tested this possibility by performing PRMT1 methyltransferase assay with GST-PXR as the substrate. In this assay, PRMT1 did not methylate the GST-PXR fusion peptide (data not shown).

The important role of PRMT1 in the transcriptional activity of PXR was further confirmed using two approaches: 1) knockdown of PRMT1 expression by PRMT1-specific siRNA drastically inhibited the PXR-regulated luciferase reporter gene activity, and 2) in PRMT1 (-/-) cells PXR transcriptional activity was not detectable, suggesting an indispensable function of PRMT1 for the PXR-regulated gene expression.

Interestingly, our results indicate that PXR also regulates the functions of PRMT1in at least two aspects. Firstly, PXR regulates the PRMT1 substrate specificity. In comparison with recombinant PRMT1, the PXR-associated PRMT1 demonstrated preference for certain pre-acetylated H4 peptides; whereas pre-acetylation of H4K12 is inhibitory to methylation of H4R3 by PRMT1 (Fig. 2.1D), acetylation of H4K5, H4K8, and H4K16 has no effect on the H4R3 methylation. Secondly, the presence of PXR has a

significant effect on the cellular compartmentalization of PRMT1. In normal human hepatocytes that express PXR, PRMT1 is primarily localized in the nucleus (data not shown); however, in HepG2 cells that lack PXR, PRMT1 is localized mostly in the cytoplasm (Fig. 2.5). Stable transfection of PXR restores the PXR responses (5, 24), and PRMT1 is localized in the nucleus as demonstrated in this study (Fig. 2.5), suggesting PXR plays an important role in PRMT1 nuclear translocation. However, it is possible that this phenomenon is unique to hepatocytes, and PXR overexpression causes PRMT1 nuclear translocation. To further analyze the role of PXR in PRMT1 nuclear translocation, we extend this observation to another cell line.We transfected PXR into colon epithelial cancer cell line HT29, which doesn't express endogenous PXR (Fig. 2.5). Similar to HepG2 cells, PRMT1 also translocated from cytoplasmic region into nucleus with PXR expression, further supporting the role of PXR in nuclear localization of PRMT1 (Fig. 2.5).

Taken together, these results suggest that interaction between PXR and PRMT1 is reciprocal and PRMT1 not only regulates PXR transcriptional activity, but PXR also regulates the activity of PRMT1 through controlling its cellular compartmentalization in addition to substrate preferences. The effects of PXR on PRMT1 suggest that PXR has a rather general effect on the cellular processes that require PRMT1 and furthermore, the function of PXR may go beyond the xenobiotic/drug metabolism to include many aspects of physiological/pathophysiological processes which require PRMT1.

CHAPTER III

PRETREATMENT WITH DMSO GIVES A PRIMING EFFECT ON PXR TRANSACTIVATION

3.1 OVERVIEW

Prior exposure to chemicals/agents may alter epigenome in such a way that subsequent exposure to the same or different xenobiotic would produce different response. This phenomenon has been observed in a differentiation study of leukemia HL-60 cells: Pre-exposure to 1-2% dimethyl sulfoxide (DMSO) for 24 h caused much more rapid differentiation upon 9-*cis*-retinoic acid stimulation than 9-*cis*-retinoic acid treatment alone (323).

In this study, we demonstrated a similar "priming" effect for PXR-regulated gene expression. Upon pre-exposure to 1.25-2.5% DMSO, PXR-mediated *CYP3A4* induction by rifampicin was significantly increased campared to "naïve" cells, which were not pre-exposed. Obviously, understanding the underpinning mechanism of this "priming" effect has important implications for xenobiotic/drug metabolism and detoxification, drug-drug interactions as well as therapeutic application of drug combinations.

Post-translational modifications on the N-termini of histones have been shown to play critical roles in gene regulation. In our previous study, PRMT1 (protein arginine methyltransferase 1) was demonstrated to be an essential coactivator for PXR-mediated gene regulation. In this study, PRMT1 was also determined to play an important role in the priming process. The priming effect was enhanced by PRMT1 overexpression and

abolished when PRMT1 was knocked down. Inhibition of methyltransferase activity by ADOX before priming also inhibited the priming effect while ADOX treatment after priming had no effect.

The combination and interplays of histone modifications has been shown to create a marking system ("histone code"), which is part of the epigenetic mechanisms for gene regulation (205). Recent evidence showed that modification marks usually prevent the adjacent arginine to be methylated. For example, H3K9 acetylation inhibits H3R8 methylation (298). H3R8 methylation inhibits H3K9 methylation (312). H4R3 methylation by PRMT1 was reported to set the stage for subsequent histone modifications *in vivo* (286) and facilitate H4 acetylation by p300 *in vitro* (50). However, acetylation of H4 inhibits recombinant PRMT1 methylation on H4R3 (50). In our previous study, preacetylation of H4K12 but not lysines 5, 8 and 16 inhibited PXR-associated PRMT1 methylation of H4R3 in an *in vitro* histone methyltransferase assay. In this study, we observed an *in vivo* decrease of H4K12 acetylation in the *CYP3A4* promoter region upon rifampicin or priming stimulation. This observation also fit our hypothesis that, to complete the transcription cycle, specific acetylation has to be removed to allow H4R3 methylation, which facilitates total histone acetylation.

Treatment with 2% DMSO for 24 h also induced a G0/G1 arrest in HepG2 cells regardless of PXR expression. This observation suggested that 24 h treatment of 2% DMSO not only alters histone modifications on regulatory region of selective genes, but also exhibits a global impact on cellular physiological process.

3.2 MATERIALS AND METHODS

3.2.1 *Materials*

DMSO, rifampicin, TSA, ADOX and anti-FLAG M2 antibody are from Sigma. Anti-acetyl-(pan)H4, anti-acetyl-H3, anti-acetyl-H4K8, and anti-acetyl-H4K12 antibodies were purchased from Upstate (Millipore, Billerica, MA). Anti-methyl-H4R3 is from Abcam (Cambridge, MA). Isotype IgGs were from Santa Cruz Biotechnology (Santa Cruz, CA).

3.2.2 *Cells*

HepG2 and HepG2 derived cells were cultured in DMEM (Dulbecco's modified Eagle's medium) (HyClone, Logan, UT) supplemented with 10% FBS (fetal bovine serum) (Sigma) and 1x antibiotic and antimycotic (Invitrogen, Carlsbad, CA). PXR-HepG2 and reporter-HepG2 stable transfectants were created as described in (318). In brief, HepG2 cells are stably transfected with 3x Flag-PXR plasmid to create PXR-HepG2 cells and cotransfected with neo-PXR and PXR-directed reporter plasmid pGL3-*3A4*-Luc (318) to create reporter-HepG2 cells.

Cell suspensions of primary human hepatocytes was purchased from Cambrex BioScience (Walkersville, MD). Upon arrival, the cells were resuspended in DMEM medium supplemented with 5% FBS, 1x antibiotic and antimycotic, 4 μg/ml insulin, and 1μM dexamethasone, and plated in collagen coated 12-well plates. After seeded, cells were maintained in WEM containing ITS+, 0.1 μM dexamethasone and 1x antibiotic overnight.

3.2.3 *Transient transfection and Luciferase assay*

Cells were seeded in 12-well plates. When growth reached 50% confluence, cells were transfected with plasmid DNA for 12 h using Lipofectamine (Invitrogen). The transfected cells were treated with chemicals or vehicle accordingly. The luciferase assay was performed using a luciferase assay system kit, according to the manufacturer's recommendation (Promega).

3.2.4 *Statistical analysis*

Statistical evaluations were conducted using two-tailed *t* test with triplicates for each treatment. A *p* value of less than 0.01 was considered to be statistically significant. Data are the means $\pm$ S.D. of three independent results.

3.2.5 *Real time PCR*

Total RNA was extracted using the Trizol reagent according to the manual (Invitrogen). Reverse-transcription was performed with an M-MLV reverse transcriptase kit with random primers (Invitrogen). The amount of complementary DNA template was determined by real-time quantitative PCR in triplicates with a 1-μl sample. Amplifications were performed in the 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA) with SYBR Green Master Mix (Applied Biosystems).

3.2.6 *Small interfering RNA*

The small interfering RNA-expressing plasmid were constructed by cloning the sequence targeting PRMT1 at coding region sequence 756–773 (308) (siPRMT1) into pSilencer 5.1 plasmid according to the manual (Ambion). The targeting plasmid was created by inserting 5'-GATCCGATCCACTGGTGGGAGAACTTCAAGAGAGTT

CTCCCACCAGTGGATTTTTTTGGAAAAGCT-3' (siPRMT1). The siRNA plasmid and the scramble siRNA control were transfected into PXR-reporter-HepG2 cells. The transfected cells were treated with rifampicin (10 μM, 48 h).

3.2.7 *Chromatin Immunoprecipitation (ChIP)*

ChIP assay was performed according to the manufacturer's protocol from Upstate, using the ChIP assay kit with modifications. Briefly, after treatment, cells were cross-linked with 1% formaldehyde for 15 min at room temperature, and then the reaction was stopped by incubating in glycine with a final concentration of 0.125 M for 5 min. Cells were washed three times with cold PBS and harvested by scraping with cell scraper. Then the cells were lysed in the SDS lysis buffer (1% SDS, 10 mM EDTA, and 50 mM Tris-HCl, pH 8.1) on ice for 10 min. The samples were sonicated into DNA fragments of 0.2–1 kb (checked by agarose gel electrophoresis/ethidium bromide staining) and microcentrifuged at maximal speed for 10 min at 4°C. The supernatant was precleared by rotating with 60 μl of Salmon Sperm DNA/protein-agarose slurry for 30 min at 4°C and then aliquoted after centrifugation. 20 μl was saved as input and 200 μl (equal to one-fifth the amount of cells from one 100% confluent 15-cm dish) was used for each antibody. Each 200-μl supernatant aliquot was diluted with 800 μl of ChIP dilution buffer (0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCl, pH 8.1, and 167 mM NaCl) and incubated with the specific antibody (1 μg/sample) at 4°C overnight. A mock precipitation without antibody was used as negative control. The next day, 60 μl of salmon sperm DNA/protein-agarose slurry was added to each sample and incubated at 4 °C for another 2–4 h. The beads were then washed for 3–5 min with 1 ml of each

buffers listed: low salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 150 mM NaCl), high salt wash buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris-HCl, pH 8.1, 500 mM NaCl), and LiCl wash buffer (0.25 M LiCl, 1% IGEPAL-CA630, 1% deoxycholic acid (sodium salt), 1 mM EDTA, 10 mM Tris-HCl, pH 8.1). After all washes, pellets were suspended by vertex with 150 μl of freshly prepared elution buffer (0.1 M NaHCO3, 1% SDS) for 15 min, and then supernatant was collected. This elution progress was repeated once again, and in total 300 μl elutes were collected. The one-tenth input was diluted with dilution buffer to a total volume of 300 μl. Elutes and diluted inputs were incubated in 0.3 M NaCl at 65 °C for 4 h to reverse formaldehyde cross-linking. Then 10 μl of 0.5 M EDTA, 20 μl of 1 M Tris-HCl, pH 6.5, and 20 μg of proteinase K were added to the sample and incubated at 45 °C for 1 h. DNA was extracted with phenol/chloroform and then incubated with 10 μg of glycogen in 75% ethanol at -20 °C overnight. After precipitation by centrifuging at 12,000 x *g* for 30 min at 4 °C, the recovered DNA pellets were dissolved in 30 μl of distilled water. The DNA target in the sample was determined by PCR.

3.2.8 *Cell cycle analyzed by flow cytometry*

After treatment, cells were harvested with trypsinization and fixed in 70% ethanol for 2 h. After washing with PBS, cells were resuspended in Propidium Iodide/PBS staining solution (2 μg/ml) supplemented with 0.1 mg/ml RNase and incubated for 30 min at room temperature. Flow cytometry analysis was performed with an FACS Calibur flow cytometer (Becton Dickinson) with the excitation at 488 nm and the emission at 520 nm.

3.2.9 *GST pull-down assay*

The GST pull-down assay was performed as described (52). Briefly, [35S]methionine-labeled fulllength PRMT1 protein was generated with a TNT-coupled Reticulocyte Lysate System (Promega) using the SP6 promoter-driven cDNA plasmid as the template. PCR-generated RXR cDNA fragments were inserted in-frame into pGEX-5X-3 (Amersham Biosciences). The plasmids were expressed in *Escherichia coli* (BL21), and fusion polypeptides were purified with glutathione-Sepharose 4B beads (Amersham Biosciences) according to the manufacturer's instruction. Twenty micrograms of each fusion polypeptide (estimated by comparison with bovine serum albumin in an SDS-PAGE gel with Coomassie Blue staining) was incubated with 20 μl of radiolabeled PRMT1 in a total volume of 200 μl of binding reaction buffer (20 mM Hepes pH 7.9, 1% Triton X-100, 20 mM dithiothreitol, 0.5% bovine serum albumin, and 100 mM KCl) for 3 h at 4 °C. After incubation, beads were washed three times with the same buffer without bovine serum albumin. The bound proteins were eluted by boiling in the SDS-PAGE sample buffer and resolved by 12% SDS-PAGE gel electrophoresis. The signals were detected by autoradiography. The input control was 2 μl of the radioactive PRMT1.

3.3 RESULTS

3.3.1 *Pretreatment of DMSO increased the PXR transactivatity upon ligand treatment.*

To investigate the "priming" effect on PXR-regulated gene expression, we utilized HepG2 cells stably transfected with human PXR and *CYP3A4* driven luciferase reporter gene (reporter-HepG2) to analyze the effect of DMSO on rifampicin (RIF) induced PXR transcriptional activity. Pretreatment with 1.25% or 2.5% DMSO overnight significantly increased PXR-regulated *CYP3A4*-luciferase activation by rifampicin (Fig. 3.1A).

We also investigated this "priming" effect in primary human hepatocytes. Upon rifampicin activation, CYP3A4 mRNA level was also significantly elevated when cells were pretreated with 1.25% or 2.5% DMSO (Fig. 3.1B). The effect decreased at a higher dose namly, 5% for the cell line and 2.5% for primary hepatocytes. That might be due to the susceptibility difference between the cell line and the primary hepatocytes.

In order to characterize the priming effects, we tested different pre-exposure times for DMSO. Pretreatment with DMSO for 9 h gave a significant "priming" effect. As the pre-exposure to DMSO was prolonged, the priming effect was enhanced (Fig. 3.2A), indicating that priming is not only dose- but also time-dependent.

We performed another experiment in which cells were pretreated with 2.5% DMSO for 18 h. DMSO was then withdrawn and rifampicin was added immediately or after a certain period (24, 48 or 72 h). After a 48 h interval, the priming effect was still significant (Fig 3.2B, compare second and the fourth lanes of 48 h); however, after 72 h, primed and naive cells had no significant difference in PXR target gene induction. In conclusion, the priming effect can persist for up to 48 h.

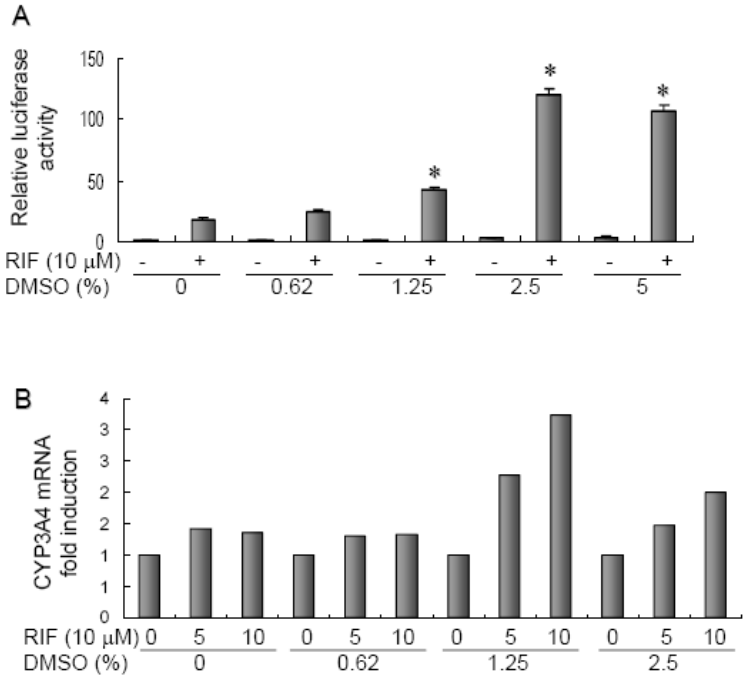


Fig. 3. 1. DMSO pretreatment enhanced the PXR transcriptional activity. (A) HepG2 cells were stably transfected with PXR and *CYP3A4*-luciferase reporter gene. Cells were exposed to DMSO for 18 h. DMSO was withdrawn and cells were exposed to 10 μM RIF. Luciferase activity was measured 48 h later. *, statistically significant difference (p<0.05). The data are the means ± S.D. of three independent measurements. (B) Human primary hepatocytes were seeded in 12 well plates. Cells were treated with DMSO and after 18 h DMSO was withdrawn. Cells were exposed to 10 μM RIF. *Cyp3a4* mRNA level was measured 24 h later by real-time quantitative RT-PCR.

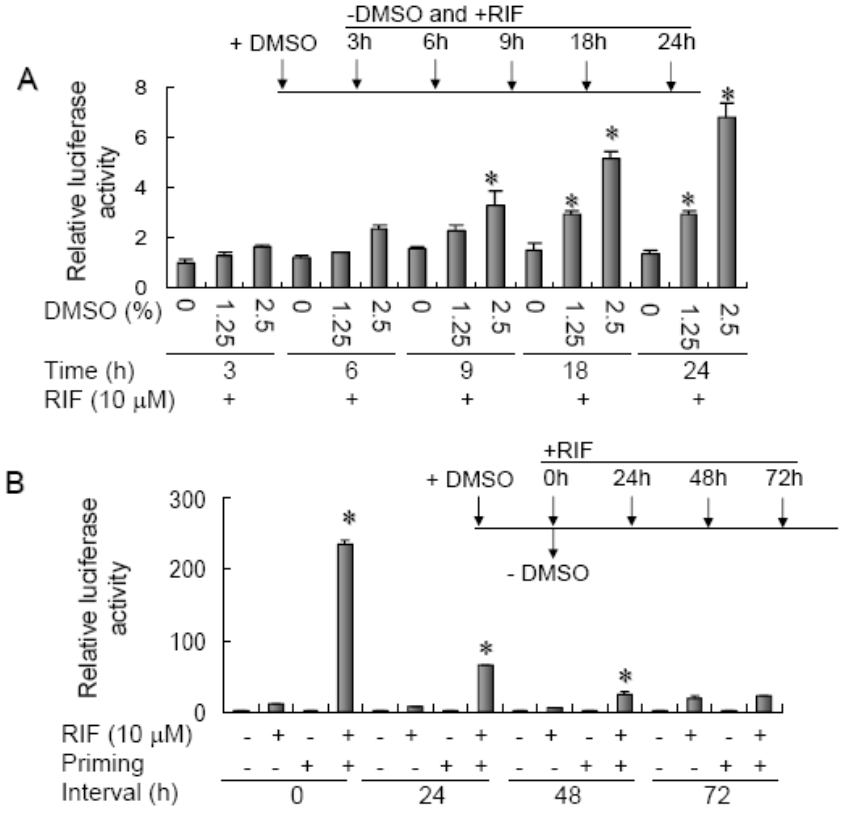


Fig. 3.2. Priming was time and dose dependent and persisted for 48 h. (A) Reporter-HepG2 cells were exposed to DMSO for indicated duration and then to 10 μM RIF without DMSO. Luciferase activity was measured 48 h later. (B) Cells were treated with 2.5% DMSO and after 24 h DMSO was withdrawn. After indicated hours, cells were treated with 10 μM RIF. Luciferase activity was measured 48 h later. *, statistically significant difference (p<0.05). The data are the means ± S.D. of three independent measurements.

3.3.2 *Pretreatment of DMSO altered histone modifications in the regulatory region of PXR target gene*

To understand the epigenetic mechanisms of the priming effect, we used PXR-HepG2 cells, that are HepG2 cells stably transfected with 3x Flag-PXR, to perform the ChIP assay. Primed and naïve cells treated with rifampicin or vehicle were harvested and ChIP assay was performed as described in the method. We analyzed several histone modifications in the *CYP3A4* proximal promoter and a distal xenobiotic response enhancer module (XREM) about 8 kb upstream of the transcriptional starting site. PXR was shown to regulate *CYP3A4* expression through binding to these regions (123). As shown in Fig. 3.3, in both regulatory regions, total acetylation of H3 and H4 correlated with target gene activation that was increased upon rifampicin treatment and further enhanced upon priming. The difference was that basal acetylation of H4 was higher than H3. As an active transcription mark essential for histone acetylation (286), methylation of H4R3 was also increased upon rifampicin and significantly enhanced upon priming.

Although total acetylation of H4 was correlated with gene activation, acetylation at selective lysines gave different correlations. Acetylation of lysine 8 was not changed by rifampicin treatment or pretreatment of DMSO. However, acetylation of lysine 12 was significantly decreased in both regions in naïve cells upon rifampicin activation. In our previous study, we detected an inhibitory effect on PXR-associated PRMT1 methylation of H4R3 by preacetylation of H4K12, while preacetylation on other lysines did not have this inhibition. Therefore, removal of H4K12 acetylation may facilitate H4R3 methylation for PXR target gene activation.

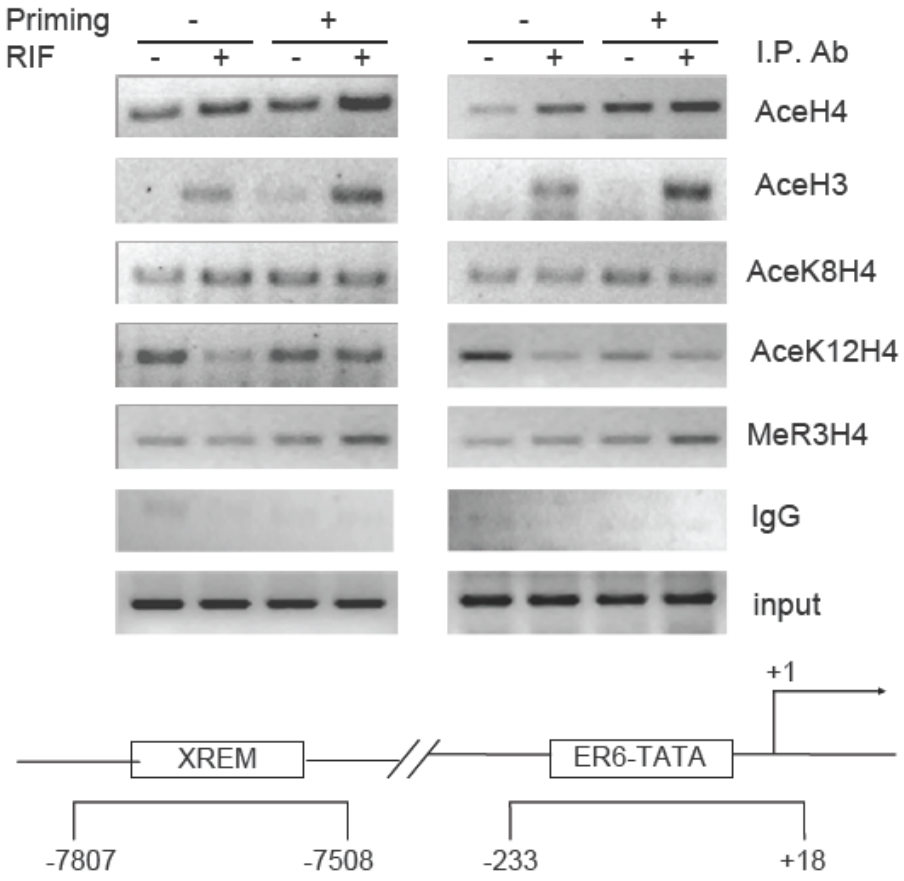


Fig. 3.3. DMSO priming altered histone modifications in the *cyp3a4* regulatory regions. PXR-HepG2 cells were treated with/without 2% DMSO for 24 h and then with 10 μM rifampicin or vehicle for 2 h. Histone modifications were analyzed by ChIP assay with indicated antibodies. XREM, xenobiotic response module; ER6-TATA, proximal promoter region.

3.3.3 *PRMT1 played an important role in the priming*

PRMT1 is the major protein arginine methyltransferase in mammalian cells which selectively methylates H4R3. In our previous study, we demonstrated that PRMT1 is an important PXR coactivator which associates with PXR *in vitro* and *in vivo*. To

investigate whether PRMT1 played a role in this PXR-mediated priming effect, we transiently transfected PRMT1 expression plasmid into the reporter-HepG2 cells. Results are shown in Fig. 3.4A. The rifampicin induction of *CYP3A4*-luciferase expression was not influenced by overexpression of PRMT1, which indicated a saturated endogenous PRMT1 level for PXR-mediated gene activation. However, the priming effect was enhanced by PRMT1 transfection in a dose-dependent manner.

We also knocked down PRMT1 by expressing small interference RNA of PRMT1 (siPRMT1) in the reporter-HepG2 cells. Knockdown of PRMT1 decreased rifampicin induction of *CYP3A4*-luciferase expression in both unprimed "naïve" and primed reporter-HepG2 cells dose-dependently (Fig. 3.4C). Transfection of a control scramble siRNA did not influence either *CYP3A4*-luciferase induction or the priming effect (Fig. 3.4D). However, the priming effect on induction of *CYP3A4* mRNA level is more sensitive to PRMT1 knockdown. Transient transfection of a moderate amount of siPRMT1 abolished the priming effect without significant decreasing induction of CYP3A4 mRNA in naïve cells (Fig. 3.4B). In conclusion, pretreatment with DMSO sensitized cells to ligand-dependent activation of PXR also to expression of the coactivator PRMT1. PRMT1, an essential PXR coactivator, is also important in this priming process.

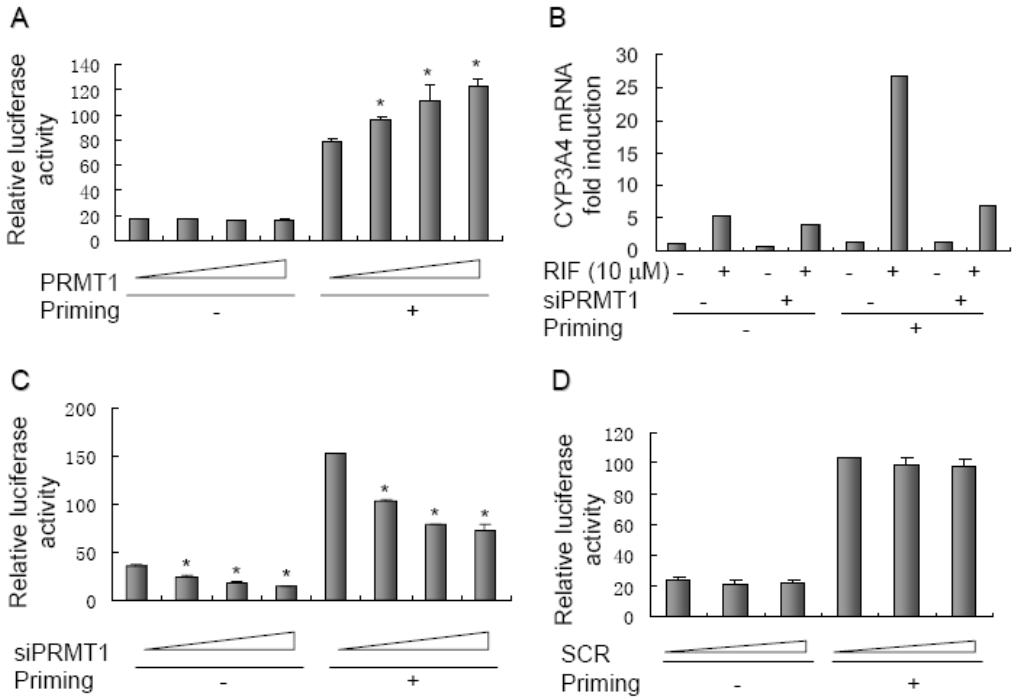


Fig. 3.4. PRMT1 played an important role in the priming effect. (A) HepG2 cells which stably express PXR and *cyp3a4*-luc were transiently transfected with PRMT1 plasmid (0, 0.1, 0.2, or 0.4 μg/ml). Transfected cells were pretreated with (primed) or without (naïve) 2% DMSO for 24 h and then exposed to rifampicin (10 μM). Luciferase activity was measured 48 h later. (B) Reporter-HepG2 cells were transiently transfected with 0.05 μg/ml siPRMT1 or scramble siRNA. Primed and naïve transfected cells were treated with 10 μM rifampicin. After 24 h, *CYP3A4* mRNA level was determined by quantitative real-time PCR. (C and D) Reporter-HepG2 cells were transiently transfected with siPRMT1 (C, 0, 0.05, 0.1, or 0.2 μg/ml) or control scrambled siRNA (D, 0, 0.1 or 0.2 μg/ml). Primed or naïve transfected cells were exposed to 10 μM rifampicin. Luciferase activity was measured 48 h later. *, statistically significant difference (p<0.05). The data are the means±S.D. of three independent measurements.

3.3.4 *Histone modifications played a critical role in the priming effect*

We demonstrated that PRMT1 played an important role in the priming and we also observed significantly elevated H4R3 methylation in primed cells treated with rifampicin. In order to investigate the importance of the methyltransferase activity for priming effect, we treated reporter-HepG2 cells with ADOX, an inhibitor of methyltransferase (274,320). As shown in Fig. 3.5, treatment with ADOX before priming inhibited the priming effect. However, treatment with ADOX after priming did not significantly change the PXR transactivation. These data indicated a critical role for methyltransferase activity. Although we demonstrated an important role for PRMT1 in the priming effect, we cannot rule out other methyltransferases in this process.

We observed reduced H4K12 acetylation in the promoter region upon DMSO pretreatment. Therefore, histone deacetylases (HDACs) may be important for the priming effect. We treated reporter-HepG2 cells with TSA, an HDAC inhibitor (Fig. 3.6). Since HDAC is inhibited, total histone acetylation was increased and TSA enhanced induction of luciferase expression by rifampicin. However, the priming effect was abolished when cells were exposed to TSA before priming. This demonstrates that HDACs played a significant role in the priming. Accumulated acetylation of H4 also inhibited H4R3 methylation, indirectly indicating the importance of H4R3 methylation. However, treatment of TSA after priming further elevated the PXR transactivation (Fig. 3.6B).

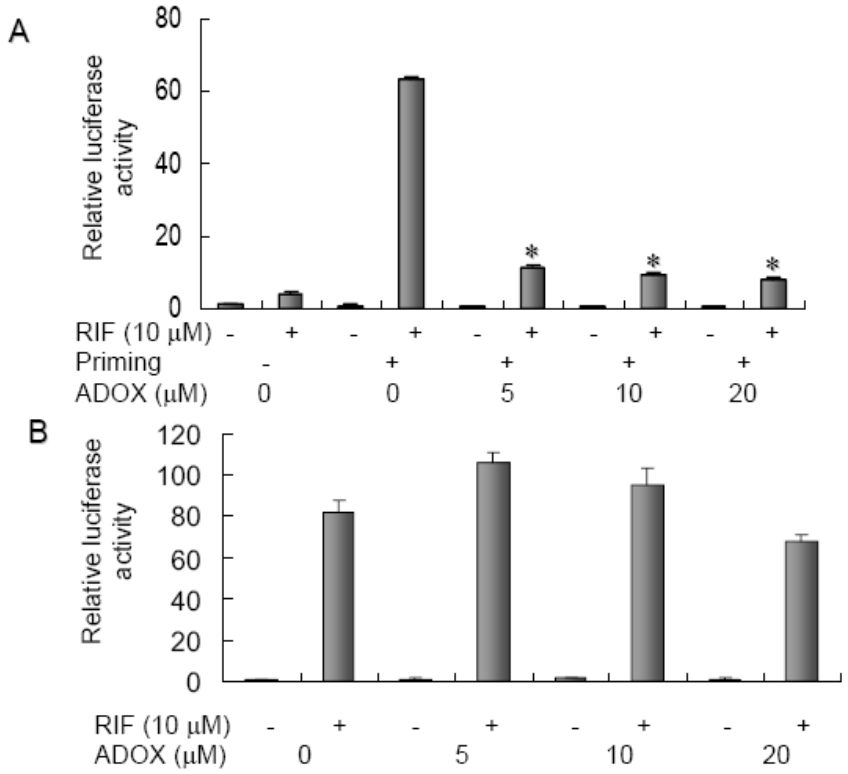


Fig. 3.5. Inhibiton of methyltransferase activity by Adox abolished the priming effect while the same treatment post priming had no impact. (A) Adox was added to reporter-HepG2 cells 24 h before priming. (B) Cells were primed with 2% DMSO for 24 h and then treated with Adox for 24 h. After these treatments, cells were treated with rifampicin. Luciferase activity was measured 48 h later. *, statistically significant difference (p<0.05). The data are the means±S.D. of three independent measurements.

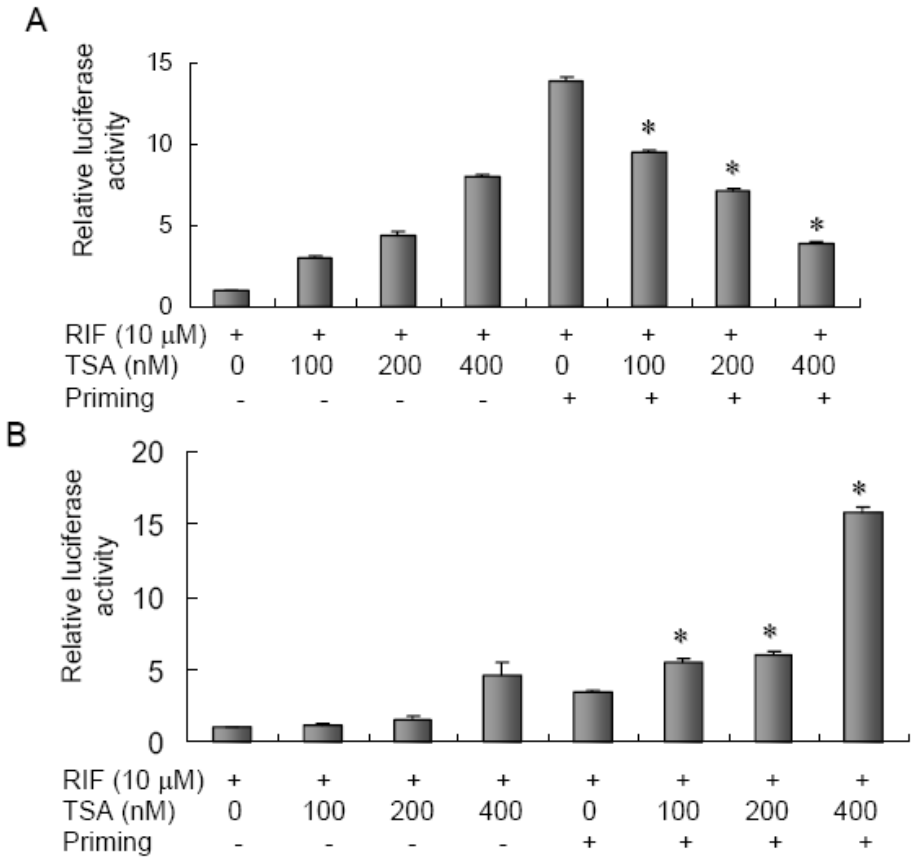


Fig. 3.6. HDAC inhibitor TSA inhibited priming effect while the same treatment post priming enhanced it. (A) Reporter-HepG2 cells were exposed to TSA 24 h before priming. (B) Cells were treated with TSA after priming. Then cells were treated with rifampicin. Luciferase activity was measured 48 h later. *, statistically significant difference (p<0.05). The data are the means±S.D. of three independent measurements.

3.3.5 *Treatment with 2% DMSO stimulated G0/G1 arrest in HepG2 cells*

In order to determine whether 2% DMSO can influence cell cycle, we stained HepG2 and reporter-HepG2 cells with propidium iodide and performed flow cytometry. As shown in Fig. 3.7, percentage of cells at G0/G1 phase was significant higher in cells

treated with 2% DMSO for 24 h compared to untreated cells in spite of their expression of PXR (82.61% v.s. 70.29% for reporter-HepG2 and 81.49% v.s. 60.69% for HepG2).

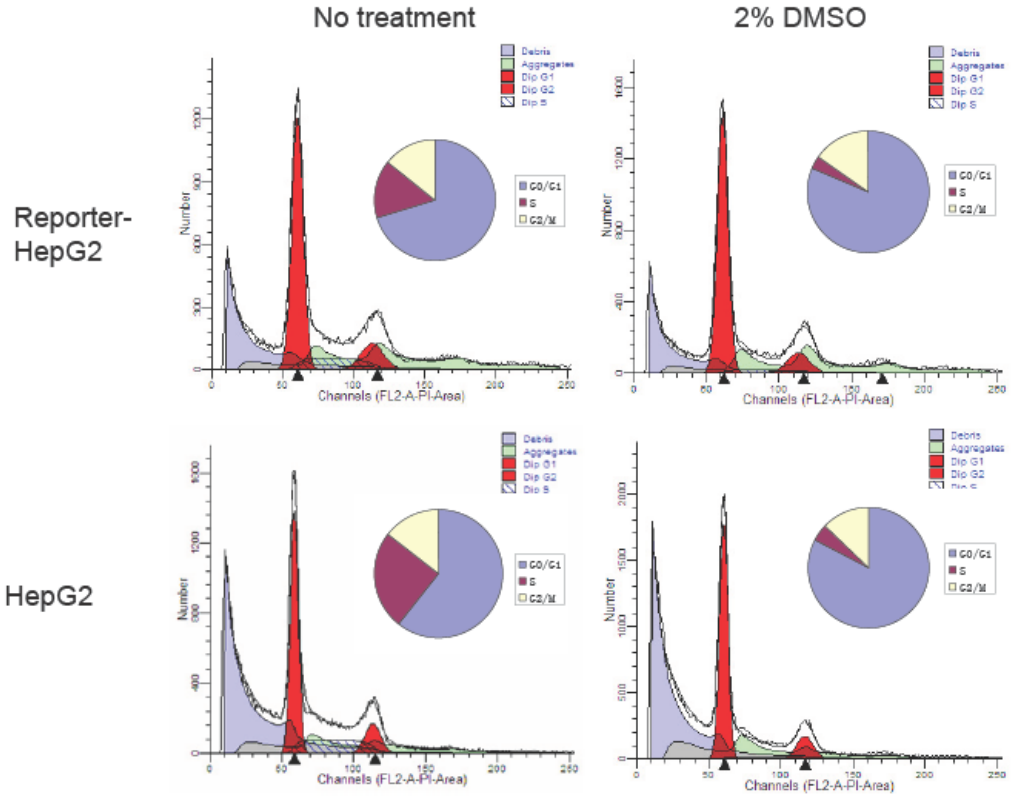


Fig. 3.7. DMSO treatment caused G0/G1 arrest. HepG2 cells with or without stably transfected PXR were treated with 2% DMSO for 24 h. Cells without treatment (naïve) were used as a control. Cells stained with Propidium Iodide (P.I.) were subjected to flowcytometry analysis.

3.4 SUMMARY AND DISCUSSION

DMSO was the first chemical discovered to induce growth arrest and terminal differentiation of murine erythroleukemia cells (324). Later studies on DMSO and other polar solvents which cause the same effect elicited discovery and development of cancer therapeutic compounds that exhibit histone deacetylase activity (325). Histone modification activity for DMSO was not reported, however, several histone modification changes were identified at RAR targeted promoters in HL-60 leukemia cells treated with 2% DMSO for 16-24 h (323).

Although the effects of DMSO on leukemia cells have been studies for over 35 years, the influence of this solvent on other cell types was not clear. DMSO is a polar solvent widely used in biological and medical science. It is a cryoprotectant for preserving cell, organs, tissues and embryos. However, the physiological effects of DMSO on exposed cells or organs have not been extensively investigated. Although DMSO has FDA-approved therapeutic applications for many inflammatory conditions, such as treatment for interstitial cystitis, not much information on its mechanism has been reported.

In this study, we demonstrated that DMSO pretreatment can “prime” the PXR-regulated gene expression resulting in increased responses to PXR ligands (Fig. 3.1). This effect was DMSO-dose and treatment duration (time) dependent (Fig. 3.2). Although high dose DMSO reduced the effect, which may be due to toxicity, the effect seemed to be enhanced as the treatment was prolonged. DMSO is usually used as a vehicle control in culture studies at the dose as low as 0.1 or 0.2%. However, when cells

were exposed to this vehicle control for long periods, some effect are observed. Human primary hepatocytes can also be sensitized by 1.25% DMSO to PXR ligand activation (Fig. 3.1B). This data provided evidence for the priming effect in primary hepatocyte and this may have important implications for drug-drug interactions or drug combination therapy.

Mechanistically, priming may involve an epigenetic mechanism through alteration of histone modifications (Fig. 3.3). The fact that the priming effect lasts longer than one mitotic division (average 24 h, ATCC) is consistent with this notion (Fig. 3.2B). Total acetylation of H4 and H3, which was increased by rifampicin, was enhanced by priming, correlated with the transcription level of *cyp3a4* expression. Methylation on H4R3 was significantly increased upon rifampicin treatment in primed cells compared to naïve cells (Fig. 3.3).

PRMT1 is a required co-regulator for PXR as demonstrated by our previous study. Methylation of H4R3 by PRMT1 has been shown to set the stage for subsequent acetylation (286). However, acetylation, in turn, inhibits H4R3 methylation (50), suggesting an intricate interaction between histone marking system (histone code) which may be a "feed-back" inhibition mechanism to fine-tune the PXR-regulated responses (317). Based on this observation, we propose a model of transcription "relay" (Fig. 8) to depict the methylation and acetylation interaction (317). Our results with the Adox and TSA treatments are also consistent with this model (Fig. 3.5 and 3.6).

The key points are the followings: 1. methylation of H4R3 by PRMT1 is critical for the priming effect, since this methylation was significantly increased upon rifampicin

stimulation after priming (Fig. 3.3). The priming effect was enhanced by overexpression of PRMT1 and inhibited by knockdown of PRMT1 as well as Adox pretreatment. However, Adox treatment had no significant effect after priming (Fig. 3.2 and 3.5). PRMT1 was also demonstrated to play an important role in the priming of leukemia HL-60 cells through RAR-mediated gene regulation. We demonstrated a direct interaction between PRMT1 and RXR by GST pull-down assay (Fig. 3.8). RXR ligand binding domain is the interactive domain binding to PRMT1. RAR and PXR both formed heterodimers with RXR to regulate target genes. We hypothesized that all nuclear receptors which form heterodimers with RXR can recruit PRMT1 and be sensitized by priming. Further verification of this hypothesis is currently being investigated. 2. The sequence of modifications is important i.e. methylation to acetylation is unidirectional and in order to complete transcription, the acetylation mark needs to be removed by HDAC. Furthermore, in our previous study, we demonstrated that H4K12 acetylation strongly inhibited the methylation of H4R3 by PXR-associated PRMT1. This is consistent with decreased H4K12 acetylation upon treatment with rifampicin and upon priming in the promoter region (Fig. 3.3). This observation also supports our transcription "relay" model that lysine acetylation needs to be removed for re-methylation of H4R3. Our results demonstrate that HDAC activity directed at a specific residue, whereas HDACs usually deacetylate multiple residues. TSA treatment before priming, prevented removal of H4K12 acetylation, and inhibited the priming effect. After H4K12 deacetylation is finished after priming, TSA treatment had no effect on H4K12 acetylation and H4R3 methylation. However, inhibition of other HDACs caused

further accumulation of H3 and H4 acetylation which correlated with higher gene activation.

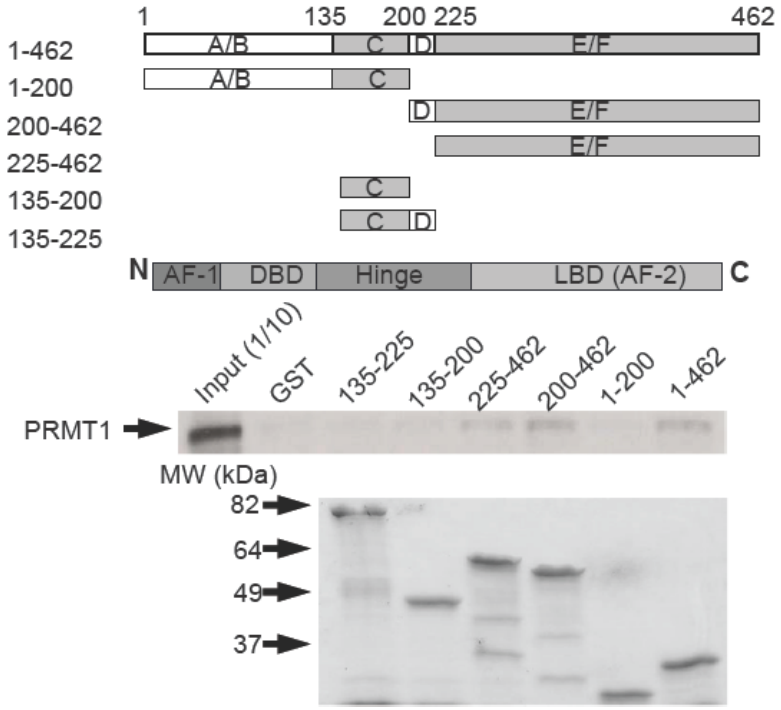


Fig. 3.8. RXR interacted with PRMT1 through its ligand binding domain. Full-length RXR and various RXR fragments were fused with GST and the fusion peptides coupled with glutathione-Sepharose beads were incubated with radiolabeled PRMT1. The precipitated complexes were analyzed by autoradiography following SDS-PAGE (middle panel). Upper panel, illustration of RXR fragments. Lower panel, loading control of the GST-fused RXR fragments (Coomassie Blue staining).

An unexpected difference on H4K12 acetylation after priming was observed between *CYP3A4* enhancer and promoter regions. Acetylation of H4K12 at the enhancer

region was significantly reduced upon treatment with rifampicin. However, this change did not occur after priming. In contrast, H4K12 acetylation at the promoter region was significantly reduced upon treatment with rifampicin or priming. In another study from our laboratory, we demonstrated a looping structure between *CYP3A4* enhancer and promoter regions upon rifampicin activation. Based on this observation, we hypothesized that without priming, rifampicin stimulated the looping structure and also a recruitment of HDAC to deacetylate H4K12. As the looping structure is formed, HDAC is able to approach the substrate on both the enhancer and promoter regions. After H4K12 is deacetylated, PRMT1 methylates H4R3 and to facilitate the subsequent acetylation of H4 and H3. However, upon priming, HDAC is recruited only to the promoter region to deacetylate H4K12. After the looping structure is formed, PRMT1 can methylate H4R3 without recruitment of the HDAC to deacetylate H4K12. Therefore, the acetylation of H4K12 at the enhancer region is not removed after priming.

Our explanation is based on the histone modification changes that were observed. There may be other histone modifications playing a role in the priming process, such as H3K4 and H3K9 methylation.

Thus 2% DMSO treatment for 24 h not only changes histone modifications on selective target gene regulatory regions, but also caused some global physiological effects, including G0/G1 arrest of HepG2 cells which was independent of PXR. This data suggests that this cell cycle regulation is global and at the level of epigenome modification.

CHAPTER IV

SUMMARY AND CONCLUSION

4.1 PRMT1 PLAYS AN IMPORTANT ROLE IN PXR MEDIATED GENE REGULATION

PXR is a ligand-dependent transcription factor, regulating gene expression of enzymes and transporters involved in xenobiotic/drug metabolism. Ligand-activated PXR binds to cognate response elements in the upstream regulatory regions of target genes, such as *CYP3A4* as heterodimer with RXR, and recruits coactivators to initiate target gene activation (123). PRMT1 regulates expression of numerous genes, including nuclear receptor-regulated transcription, through methylating histone and non-histone proteins. It is a coactivator of several nuclear receptor-mediated genes (50,316). The first objective of this study, we reported that PRMT1 is required for the transcriptional activity of PXR. Co-immunoprecipitation and histone methyltransferase assays revealed that PRMT1 is a major histone methyltransferase associated with PXR. It was reported that nuclear receptor coactivator SRC-1 recruited PRMT1 as a secondary coactivator (123). However, our study shows a direct interaction between PXR and PRMT1, determined by glutathione *S*-transferase (GST) pull-down assays. GST pull-down assay also shows that the PXR ligand-binding domain is necessary for PXR-PRMT1 interactions, which is consistent with the ligand dependent association determined by mammalian two-hybrid and coimmunoprecipitation. Coimmunoprecipitation of PRMT1 with PXR in the humanized mouse liver gives *in vivo* evidence for their association.

In eukaryotes, DNA is packaged in chromatin with histones and several nonhistone proteins. In order to recruit transcriptional machinery to initiate gene expression, chromatin needs to be remodeled and modifications of histone N-terminal tails are important for this process (203). These modifications include acetylation, methylation, phosphorylation, and ubiquitylation. Acetylation of histones is correlated to gene activation (209,219), whereas methylation of histone lysine residues is related to both gene activation and corepression (249,252,256). Arginine methylation of histones also plays role in gene activation or repression through methylating N-terminal tails of histones in the promoter or other regulatory regions (50,205,278,294,295). In our study, the chromatin immunoprecipitation (ChIP) assay shows that PRMT1 is recruited to the regulatory region of the PXR target gene *CYP3A4*, with a concomitant methylation of arginine 3 of histone H4, in response to the PXR agonist rifampicin. In mammalian cells, small interfering RNA (siRNA) knockdown and gene deletion of PRMT1 greatly diminished the transcriptional activity of PXR, suggesting an indispensable role of PRMT1 in PXR-regulated gene expression.

Interestingly, PXR appears to have a reciprocal effect on the function of PRMT1 by regulating its cellular compartmentalization as well as its substrate specificity. Without PXR, PRMT1 localized in the cytoplasm of HepG2 and HT-29 cells while PXR expression caused PRMT1 to translocate into the nucleus. Recombinant PRMT1 methylation on H4R3 is slightly inhibited by preacetylation of H4 lysine 5, 8, 12 and 16 at the same level. However, methylation of H4R3 by the PXR-associated complex is selectively inhibited by preacetylation of H4K12. Single acetylation of other lysines

does not inhibit the methylation. We did not determine whether combined acetylation causes synergistic inhibition on H4R3 methylation, which was previously reported by Wang et., al (50). This *in vitro* inhibition may have implications regarding epigenetic mechanisms of gene regulation.

Taken together, these results demonstrated mutual interactions and functional interplay between PXR and PRMT1, and this interaction may be important for the epigenetics of PXR-regulated gene expression.

4.2 PRMT1 PLAYS AN IMPORTANT ROLE IN PXR MEDIATED PRIMING EFFECT

Prior exposure to chemicals/reagents may alter subsequent response to the same or a different reagent. This hypothesis has important implications in xenobiotic/drug metabolism and detoxification, drug-drug interactions as well as therapeutic application of drug combinations.

DMSO has long been used to stimulate myeloid differentiation of leukemia cells. It was shown to induce hemoglobin expression in HL-60 leukemia cells (324). In addition, a "priming" phenomenon was demonstrated in HL-60 differentiation that HL-60 cells preexposed to 1-2% DMSO for 24 h differentiate much more rapid upon 9-*cis*-retinoic acid stimulation than 9-*cis*-retinoic acid treatment alone (326). A recent study demonstrated epigenetic changes, namely histone modification changes in RAR target gene regulatory regions during the "priming" process and have suggested the involvement of PRMT1 in the priming phenomenon (323).

In our research, we studied how priming by DMSO influences the PXR-mediated activation of CYP3A4 upon rifampicin treatment. We utilized the PXR-regulated luciferase reporter cell line (reporter-HepG2) to analyze induction of target gene expression. We have found that pretreatment of PXR-HepG2 cells or human primary hepatocytes with 1-2% DMSO greatly enhanced PXR-regulated gene expression. This priming effect is both dose- and time-dependent and it can persist up to 48 h.

Results of ChIP assays showed that DMSO decreased acetylation of histone H4 lysine 12 and increased methylation of H4 arginine 3 in the regulatory region of PXR target gene CYP3A4. PRMT1 is important in the priming process. Overexpression of PRMT1 enhanced the priming effect while knockdown of PRMT1 reduced it. Prior inhibition of methyltransferase activity by ADOX or inhibition of histone deacetylase by TSA abolished the priming effect. Based on these observations and other reports (50,286), we proposed a model of "Relay between histone methylation and acetylation on the transcription cycle" that transcription cycle begins with deacetylation of specific lysine residue, such as H4K12 which facilitates methylation of histone (e.g. H4R3). This methylation in turn facilitates the total histone acetylation.

DMSO treatment has other effects on HepG2 cells including a G0/G1 cell arrest. Other responses to DMSO are currently being investigated.

REFERENCES

1. Gronemeyer, H., and Meyer, M. E. (1991) *Ann Endocrinol (Paris)* **52**(5), 335-338

2. Kawajiri, K., and Ikuta, T. (1999) *Tanpakushitsu Kakusan Koso* **44**(15 Suppl), 2377-2383

3. Oro, A. E., Umesono, K., and Evans, R. M. (1989) *Development* **107 Suppl**, 133-140

4. Repa, J. J., and Mangelsdorf, D. J. (1999) *Curr Opin Biotechnol* **10**(6), 557-563

5. Summerbell, D., and Maden, M. (1990) *Trends Neurosci* **13**(4), 142-147

6. Escriva, H., Langlois, M. C., Mendonca, R. L., Pierce, R., and Laudet, V. (1998) *Ann N Y Acad Sci* **839**, 143-146

7. Sluder, A. E., and Maina, C. V. (2001) *Trends Genet* **17**(4), 206-213

8. Zhang, Z., Burch, P. E., Cooney, A. J., Lanz, R. B., Pereira, F. A., Wu, J., Gibbs, R. A., Weinstock, G., and Wheeler, D. A. (2004) *Genome Res* **14**(4), 580-590

9. Blumberg, B., and Evans, R. M. (1998) *Genes Dev* **12**(20), 3149-3155

10. Beato, M., Herrlich, P., and Schutz, G. (1995) *Cell* **83**(6), 851-857

11. Mangelsdorf, D. J., Thummel, C., Beato, M., Herrlich, P., Schutz, G., Umesono, K., Blumberg, B., Kastner, P., Mark, M., Chambon, P., and Evans, R. M. (1995) *Cell* **83**(6), 835-839

12. Umesono, K., Murakami, K. K., Thompson, C. C., and Evans, R. M. (1991) *Cell* **65**(7), 1255-1266

13. Klinge, C. M. (2000) *Steroids* **65**(5), 227-251

14. McKenna, N. J., and O'Malley, B. W. (2002) *Cell* **108**(4), 465-474

15. Enmark, E., and Gustafsson, J. A. (1996) *Mol Endocrinol* **10**(11), 1293-1307

16. Giguere, V. (1999) *Endocr Rev* **20**(5), 689-725

17. Jones, S. A., Moore, L. B., Shenk, J. L., Wisely, G. B., Hamilton, G. A., McKee, D. D., Tomkinson, N. C., LeCluyse, E. L., Lambert, M. H., Willson, T. M., Kliewer, S. A., and Moore, J. T. (2000) *Mol Endocrinol* **14**(1), 27-39

18. Peet, D. J., Janowski, B. A., and Mangelsdorf, D. J. (1998) *Curr Opin Genet Dev* **8**(5), 571-575

19. Wang, H., Chen, J., Hollister, K., Sowers, L. C., and Forman, B. M. (1999) *Mol Cell* **3**(5), 543-553

20. Mangelsdorf, D. J., and Evans, R. M. (1995) *Cell* **83**(6), 841-850

21. Harvey, J. L., Paine, A. J., Maurel, P., and Wright, M. C. (2000) *Drug Metab Dispos* **28**(1), 96-101

22. Germain, P., Staels, B., Dacquet, C., Spedding, M., and Laudet, V. (2006) *Pharmacol Rev* **58**(4), 685-704

23. Nakata, K., Tanaka, Y., Nakano, T., Adachi, T., Tanaka, H., Kaminuma, T., and Ishikawa, T. (2006) *Drug Metab Pharmacokinet* **21**(6), 437-457

24. Moore, J. T., Collins, J. L., and Pearce, K. H. (2006) *ChemMedChem* **1**(5), 504-523

25. McEwan, I. J. (2009) *Methods Mol Biol* **505**, 3-18

26. Kumar, R., and Thompson, E. B. (2003) *Mol Endocrinol* **17**(1), 1-10

27. Lavery, D. N., and McEwan, I. J. (2005) *Biochem J* **391**(Pt 3), 449-464

28. Lefstin, J. A., and Yamamoto, K. R. (1998) *Nature* **392**(6679), 885-888

29. Berg, J. M. (1989) *Cell* **57**(7), 1065-1068

30. Klug, A., and Schwabe, J. W. (1995) *Faseb J* **9**(8), 597-604

31. Hirst, M. A., Hinck, L., Danielsen, M., and Ringold, G. M. (1992) *Proc Natl Acad Sci U S A* **89**(12), 5527-5531

32. Knotts, T. A., Orkiszewski, R. S., Cook, R. G., Edwards, D. P., and Weigel, N. L. (2001) *J Biol Chem* **276**(11), 8475-8483

33. Lee, Y. K., Choi, Y. H., Chua, S., Park, Y. J., and Moore, D. D. (2006) *J Biol Chem* **281**(12), 7850-7855

34. Kishimoto, M., Fujiki, R., Takezawa, S., Sasaki, Y., Nakamura, T., Yamaoka, K., Kitagawa, H., and Kato, S. (2006) *Endocr J* **53**(2), 157-172

35. Xu, J., and Li, Q. (2003) *Mol Endocrinol* **17**(9), 1681-1692

36. Glass, C. K., and Rosenfeld, M. G. (2000) *Genes Dev* **14**(2), 121-141

37. Steinmetz, A. C., Renaud, J. P., and Moras, D. (2001) *Annu Rev Biophys Biomol Struct* **30**, 329-359

38. Horlein, A. J., Naar, A. M., Heinzel, T., Torchia, J., Gloss, B., Kurokawa, R., Ryan, A., Kamei, Y., Soderstrom, M., Glass, C. K., and et al. (1995) *Nature* **377**(6548), 397-404

39. Chen, J. D., and Evans, R. M. (1995) *Nature* **377**(6548), 454-457

40. Bowen, N. J., Fujita, N., Kajita, M., and Wade, P. A. (2004) *Biochim Biophys Acta* **1677**(1-3), 52-57

41. Lavinsky, R. M., Jepsen, K., Heinzel, T., Torchia, J., Mullen, T. M., Schiff, R., Del-Rio, A. L., Ricote, M., Ngo, S., Gemsch, J., Hilsenbeck, S. G., Osborne, C. K., Glass, C. K., Rosenfeld, M. G., and Rose, D. W. (1998) *Proc Natl Acad Sci U S A* **95**(6), 2920-2925

42. Lonard, D. M., and O'Malley, B. W. (2005) *Trends Biochem Sci* **30**(3), 126-132

43. Fondell, J. D., Ge, H., and Roeder, R. G. (1996) *Proc Natl Acad Sci U S A* **93**(16), 8329-8333

44. Wang, C., Fu, M., Angeletti, R. H., Siconolfi-Baez, L., Reutens, A. T., Albanese, C., Lisanti, M. P., Katzenellenbogen, B. S., Kato, S., Hopp, T., Fuqua, S. A., Lopez, G. N., Kushner, P. J., and Pestell, R. G. (2001) *J Biol Chem* **276**(21), 18375-18383

45. Knutti, D., and Kralli, A. (2001) *Trends Endocrinol Metab* **12**(8), 360-365

46. Pascual-Le Tallec, L., Simone, F., Viengchareun, S., Meduri, G., Thirman, M. J., and Lombes, M. (2005) *Mol Endocrinol* **19**(5), 1158-1169

47. Savkur, R. S., and Burris, T. P. (2004) *J Pept Res* **63**(3), 207-212

48. Brown, C. E., Lechner, T., Howe, L., and Workman, J. L. (2000) *Trends Biochem Sci* **25**(1), 15-19

49. Chen, D., Huang, S. M., and Stallcup, M. R. (2000) *J Biol Chem* **275**(52), 40810-40816

50. Wang, H., Huang, Z. Q., Xia, L., Feng, Q., Erdjument-Bromage, H., Strahl, B. D., Briggs, S. D., Allis, C. D., Wong, J., Tempst, P., and Zhang, Y. (2001) *Science* **293**(5531), 853-857

51. Hebbar, P. B., and Archer, T. K. (2003) *Chromosoma* **111**(8), 495-504

52. Tian, Y., Ke, S., Chen, M., and Sheng, T. (2003) *J Biol Chem* **278**(45), 44041-44048

53. Auboeuf, D., Dowhan, D. H., Li, X., Larkin, K., Ko, L., Berget, S. M., and O'Malley, B. W. (2004) *Mol Cell Biol* **24**(1), 442-453

54. Dowhan, D. H., Hong, E. P., Auboeuf, D., Dennis, A. P., Wilson, M. M., Berget, S. M., and O'Malley, B. W. (2005) *Mol Cell* **17**(3), 429-439

55. Baek, S. H., and Rosenfeld, M. G. (2004) *Biochem Biophys Res Commun* **319**(3), 707-714

56. Hassig, C. A., and Schreiber, S. L. (1997) *Curr Opin Chem Biol* **1**(3), 300-308

57. Lee, D. Y., Teyssier, C., Strahl, B. D., and Stallcup, M. R. (2005) *Endocr Rev* **26**(2), 147-170

58. McKenna, N. J., Xu, J., Nawaz, Z., Tsai, S. Y., Tsai, M. J., and O'Malley, B. W. (1999) *J Steroid Biochem Mol Biol* **69**(1-6), 3-12

59. Schmidt, D., and Muller, S. (2003) *Cell Mol Life Sci* **60**(12), 2561-2574

60. Shao, W., Keeton, E. K., McDonnell, D. P., and Brown, M. (2004) *Proc Natl Acad Sci U S A* **101**(32), 11599-11604

61. Wu, R. C., Smith, C. L., and O'Malley, B. W. (2005) *Endocr Rev* **26**(3), 393-399

62. Lonard, D. M., and O'Malley B, W. (2007) *Mol Cell* **27**(5), 691-700

63. Chen, H., Lin, R. J., Xie, W., Wilpitz, D., and Evans, R. M. (1999) *Cell* **98**(5), 675-686

64. Bannister, A. J., Miska, E. A., Gorlich, D., and Kouzarides, T. (2000) *Curr Biol* **10**(8), 467-470

65. Soutoglou, E., Katrakili, N., and Talianidis, I. (2000) *Mol Cell* **5**(4), 745-751

66. Fu, M., Rao, M., Wang, C., Sakamaki, T., Wang, J., Di Vizio, D., Zhang, X., Albanese, C., Balk, S., Chang, C., Fan, S., Rosen, E., Palvimo, J. J., Janne, O. A., Muratoglu, S., Avantaggiati, M. L., and Pestell, R. G. (2003) *Mol Cell Biol* **23**(23), 8563-8575

67. Xu, W., Chen, H., Du, K., Asahara, H., Tini, M., Emerson, B. M., Montminy, M., and Evans, R. M. (2001) *Science* **294**(5551), 2507-2511

68. Barrero, M. J., and Malik, S. (2006) *Mol Cell* **24**(2), 233-243

69. Fan, W., Yanase, T., Wu, Y., Kawate, H., Saitoh, M., Oba, K., Nomura, M., Okabe, T., Goto, K., Yanagisawa, J., Kato, S., Takayanagi, R., and Nawata, H. (2004) *Mol Endocrinol* **18**(1), 127-141

70. Chawla, S., Hardingham, G. E., Quinn, D. R., and Bading, H. (1998) *Science* **281**(5382), 1505-1509

71. Fan, M., Rhee, J., St-Pierre, J., Handschin, C., Puigserver, P., Lin, J., Jaeger, S., Erdjument-Bromage, H., Tempst, P., and Spiegelman, B. M. (2004) *Genes Dev* **18**(3), 278-289

72. Moren, A., Hellman, U., Inada, Y., Imamura, T., Heldin, C. H., and Moustakas, A. (2003) *J Biol Chem* **278**(35), 33571-33582

73. Boudjelal, M., Wang, Z., Voorhees, J. J., and Fisher, G. J. (2000) *Cancer Res* **60**(8), 2247-2252

74. Dace, A., Zhao, L., Park, K. S., Furuno, T., Takamura, N., Nakanishi, M., West, B. L., Hanover, J. A., and Cheng, S. (2000) *Proc Natl Acad Sci U S A* **97**(16), 8985-8990

75. Deroo, B. J., Rentsch, C., Sampath, S., Young, J., DeFranco, D. B., and Archer, T. K. (2002) *Mol Cell Biol* **22**(12), 4113-4123

76. Floyd, Z. E., and Stephens, J. M. (2002) *J Biol Chem* **277**(6), 4062-4068

77. Lin, H. K., Wang, L., Hu, Y. C., Altuwaijri, S., and Chang, C. (2002) *Embo J* **21**(15), 4037-4048

78. Grossman, S. R., Deato, M. E., Brignone, C., Chan, H. M., Kung, A. L., Tagami, H., Nakatani, Y., and Livingston, D. M. (2003) *Science* **300**(5617), 342-344

79. Lehembre, F., Badenhorst, P., Muller, S., Travers, A., Schweisguth, F., and Dejean, A. (2000) *Mol Cell Biol* **20**(3), 1072-1082

80. Nishida, T., and Yasuda, H. (2002) *J Biol Chem* **277**(44), 41311-41317

81. Le Drean, Y., Mincheneau, N., Le Goff, P., and Michel, D. (2002) *Endocrinology* **143**(9), 3482-3489

82. Tian, S., Poukka, H., Palvimo, J. J., and Janne, O. A. (2002) *Biochem J* **367**(Pt 3), 907-911

83. Chauchereau, A., Amazit, L., Quesne, M., Guiochon-Mantel, A., and Milgrom, E. (2003) *J Biol Chem* **278**(14), 12335-12343

84. Kotaja, N., Karvonen, U., Janne, O. A., and Palvimo, J. J. (2002) *J Biol Chem* **277**(33), 30283-30288

85. Yang, S. H., and Sharrocks, A. D. (2004) *Mol Cell* **13**(4), 611-617

86. Girdwood, D., Bumpass, D., Vaughan, O. A., Thain, A., Anderson, L. A., Snowden, A. W., Garcia-Wilson, E., Perkins, N. D., and Hay, R. T. (2003) *Mol Cell* **11**(4), 1043-1054

87. Kirsh, O., Seeler, J. S., Pichler, A., Gast, A., Muller, S., Miska, E., Mathieu, M., Harel-Bellan, A., Kouzarides, T., Melchior, F., and Dejean, A. (2002) *Embo J* **21**(11), 2682-2691

88. Bertilsson, G., Heidrich, J., Svensson, K., Asman, M., Jendeberg, L., Sydow-Backman, M., Ohlsson, R., Postlind, H., Blomquist, P., and Berkenstam, A. (1998) *Proc Natl Acad Sci U S A* **95**(21), 12208-12213

89. Blumberg, B., Sabbagh, W., Jr., Juguilon, H., Bolado, J., Jr., van Meter, C. M., Ong, E. S., and Evans, R. M. (1998) *Genes Dev* **12**(20), 3195-3205

90. Kliewer, S. A., Moore, J. T., Wade, L., Staudinger, J. L., Watson, M. A., Jones, S. A., McKee, D. D., Oliver, B. B., Willson, T. M., Zetterstrom, R. H., Perlmann, T., and Lehmann, J. M. (1998) *Cell* **92**(1), 73-82

91. Savas, U., Wester, M. R., Griffin, K. J., and Johnson, E. F. (2000) *Drug Metab Dispos* **28**(5), 529-537

92. Zhang, H., LeCulyse, E., Liu, L., Hu, M., Matoney, L., Zhu, W., and Yan, B. (1999) *Arch Biochem Biophys* **368**(1), 14-22

93. Lamba, V., Yasuda, K., Lamba, J. K., Assem, M., Davila, J., Strom, S., and Schuetz, E. G. (2004) *Toxicol Appl Pharmacol* **199**(3), 251-265

94. Masuyama, H., Hiramatsu, Y., Mizutani, Y., Inoshita, H., and Kudo, T. (2001) *Mol Cell Endocrinol* **172**(1-2), 47-56

95. Vyhlidal, C. A., Gaedigk, R., and Leeder, J. S. (2006) *Drug Metab Dispos* **34**(1), 131-137

96. Bauer, B., Hartz, A. M., Fricker, G., and Miller, D. S. (2004) *Mol Pharmacol* **66**(3), 413-419

97. Pascussi, J. M., Drocourt, L., Fabre, J. M., Maurel, P., and Vilarem, M. J. (2000) *Mol Pharmacol* **58**(2), 361-372

98. Kamiya, A., Inoue, Y., and Gonzalez, F. J. (2003) *Hepatology* **37**(6), 1375-1384

99. Pascussi, J. M., Gerbal-Chaloin, S., Pichard-Garcia, L., Daujat, M., Fabre, J. M., Maurel, P., and Vilarem, M. J. (2000) *Biochem Biophys Res Commun* **274**(3), 707-713

100. Ding, X., and Staudinger, J. L. (2005) *Biochem Pharmacol* **69**(5), 867-873

101. Phillips, A., Hood, S. R., Gibson, G. G., and Plant, N. J. (2005) *Drug Metab Dispos* **33**(2), 233-242

102. Squires, E. J., Sueyoshi, T., and Negishi, M. (2004) *J Biol Chem* **279**(47), 49307-49314

103. Kawana, K., Ikuta, T., Kobayashi, Y., Gotoh, O., Takeda, K., and Kawajiri, K. (2003) *Mol Pharmacol* **63**(3), 524-531

104. Koyano, S., Kurose, K., Saito, Y., Ozawa, S., Hasegawa, R., Komamura, K., Ueno, K., Kamakura, S., Kitakaze, M., Nakajima, T., Matsumoto, K., Akasawa, A., Saito, H., and Sawada, J. (2004) *Drug Metab Dispos* **32**(1), 149-154

105. Bosch, T. M., Deenen, M., Pruntel, R., Smits, P. H., Schellens, J. H., Beijnen, J. H., and Meijerman, I. (2006) *Eur J Clin Pharmacol* **62**(5), 395-399

106. Hustert, E., Zibat, A., Presecan-Siedel, E., Eiselt, R., Mueller, R., Fuss, C., Brehm, I., Brinkmann, U., Eichelbaum, M., Wojnowski, L., and Burk, O. (2001) *Drug Metab Dispos* **29**(11), 1454-1459

107. King, C. R., Xiao, M., Yu, J., Minton, M. R., Addleman, N. J., Van Booven, D. J., Kwok, P. Y., McLeod, H. L., and Marsh, S. (2007) *Eur J Clin Pharmacol* **63**(6), 547-554

108. Koyano, S., Kurose, K., Ozawa, S., Saeki, M., Nakajima, Y., Hasegawa, R., Komamura, K., Ueno, K., Kamakura, S., Nakajima, T., Saito, H., Kimura, H., Goto, Y., Saitoh, O., Katoh, M., Ohnuma, T., Kawai, M., Sugai, K., Ohtsuki, T., Suzuki, C., Minami, N., Saito, Y., and Sawada, J. (2002) *Drug Metab Pharmacokinet* **17**(6), 561-565

109. Lim, Y. P., Liu, C. H., Shyu, L. J., and Huang, J. D. (2005) *Pharmacogenet Genomics* **15**(5), 337-341

110. Zhang, J., Kuehl, P., Green, E. D., Touchman, J. W., Watkins, P. B., Daly, A., Hall, S. D., Maurel, P., Relling, M., Brimer, C., Yasuda, K., Wrighton, S. A.,

Hancock, M., Kim, R. B., Strom, S., Thummel, K., Russell, C. G., Hudson, J. R., Jr., Schuetz, E. G., and Boguski, M. S. (2001) *Pharmacogenetics* **11**(7), 555-572

111. Chrencik, J. E., Orans, J., Moore, L. B., Xue, Y., Peng, L., Collins, J. L., Wisely, G. B., Lambert, M. H., Kliewer, S. A., and Redinbo, M. R. (2005) *Mol Endocrinol* **19**(5), 1125-1134

112. Watkins, R. E., Davis-Searles, P. R., Lambert, M. H., and Redinbo, M. R. (2003) *J Mol Biol* **331**(4), 815-828

113. Watkins, R. E., Maglich, J. M., Moore, L. B., Wisely, G. B., Noble, S. M., Davis-Searles, P. R., Lambert, M. H., Kliewer, S. A., and Redinbo, M. R. (2003) *Biochemistry* **42**(6), 1430-1438

114. Watkins, R. E., Wisely, G. B., Moore, L. B., Collins, J. L., Lambert, M. H., Williams, S. P., Willson, T. M., Kliewer, S. A., and Redinbo, M. R. (2001) *Science* **292**(5525), 2329-2333

115. Ostberg, T., Bertilsson, G., Jendeberg, L., Berkenstam, A., and Uppenberg, J. (2002) *Eur J Biochem* **269**(19), 4896-4904

116. Tirona, R. G., Leake, B. F., Podust, L. M., and Kim, R. B. (2004) *Mol Pharmacol* **65**(1), 36-44

117. LeCluyse, E. L. (2001) *Chem Biol Interact* **134**(3), 283-289

118. Nelson, D. R., Koymans, L., Kamataki, T., Stegeman, J. J., Feyereisen, R., Waxman, D. J., Waterman, M. R., Gotoh, O., Coon, M. J., Estabrook, R. W., Gunsalus, I. C., and Nebert, D. W. (1996) *Pharmacogenetics* **6**(1), 1-42

119. Barwick, J. L., Quattrochi, L. C., Mills, A. S., Potenza, C., Tukey, R. H., and Guzelian, P. S. (1996) *Mol Pharmacol* **50**(1), 10-16

120. Masuyama, H., Hiramatsu, Y., Kunitomi, M., Kudo, T., and MacDonald, P. N. (2000) *Mol Endocrinol* **14**(3), 421-428

121. Takeshita, A., Koibuchi, N., Oka, J., Taguchi, M., Shishiba, Y., and Ozawa, Y. (2001) *Eur J Endocrinol* **145**(4), 513-517

122. Xie, W., Barwick, J. L., Downes, M., Blumberg, B., Simon, C. M., Nelson, M. C., Neuschwander-Tetri, B. A., Brunt, E. M., Guzelian, P. S., and Evans, R. M. (2000) *Nature* **406**(6794), 435-439

123. Goodwin, B., Hodgson, E., and Liddle, C. (1999) *Mol Pharmacol* **56**(6), 1329-1339

124. Schuetz, E. G., Brimer, C., and Schuetz, J. D. (1998) *Mol Pharmacol* **54**(6), 1113-1117

125. Wright, M. C. (1999) *Biochem Soc Trans* **27**(4), 387-391

126. Desai, P. B., Nallani, S. C., Sane, R. S., Moore, L. B., Goodwin, B. J., Buckley, D. J., and Buckley, A. R. (2002) *Drug Metab Dispos* **30**(5), 608-612

127. Dussault, I., Lin, M., Hollister, K., Wang, E. H., Synold, T. W., and Forman, B. M. (2001) *J Biol Chem* **276**(36), 33309-33312

128. Tabb, M. M., Sun, A., Zhou, C., Grun, F., Errandi, J., Romero, K., Pham, H., Inoue, S., Mallick, S., Lin, M., Forman, B. M., and Blumberg, B. (2003) *J Biol Chem* **278**(45), 43919-43927

129. Xie, W., Radominska-Pandya, A., Shi, Y., Simon, C. M., Nelson, M. C., Ong, E. S., Waxman, D. J., and Evans, R. M. (2001) *Proc Natl Acad Sci U S A* **98**(6), 3375-3380

130. Moore, L. B., Parks, D. J., Jones, S. A., Bledsoe, R. K., Consler, T. G., Stimmel, J. B., Goodwin, B., Liddle, C., Blanchard, S. G., Willson, T. M., Collins, J. L., and Kliewer, S. A. (2000) *J Biol Chem* **275**(20), 15122-15127

131. Staudinger, J. L., Goodwin, B., Jones, S. A., Hawkins-Brown, D., MacKenzie, K. I., LaTour, A., Liu, Y., Klaassen, C. D., Brown, K. K., Reinhard, J., Willson, T. M., Koller, B. H., and Kliewer, S. A. (2001) *Proc Natl Acad Sci U S A* **98**(6), 3369-3374

132. Sonoda, J., Chong, L. W., Downes, M., Barish, G. D., Coulter, S., Liddle, C., Lee, C. H., and Evans, R. M. (2005) *Proc Natl Acad Sci U S A* **102**(6), 2198-2203

133. Stedman, C. A., Liddle, C., Coulter, S. A., Sonoda, J., Alvarez, J. G., Moore, D. D., Evans, R. M., and Downes, M. (2005) *Proc Natl Acad Sci U S A* **102**(6), 2063-2068

134. Gaster, B., and Holroyd, J. (2000) *Arch Intern Med* **160**(2), 152-156

135. Moore, L. B., Goodwin, B., Jones, S. A., Wisely, G. B., Serabjit-Singh, C. J., Willson, T. M., Collins, J. L., and Kliewer, S. A. (2000) *Proc Natl Acad Sci U S A* **97**(13), 7500-7502

136. Wentworth, J. M., Agostini, M., Love, J., Schwabe, J. W., and Chatterjee, V. K. (2000) *J Endocrinol* **166**(3), R11-16

137. Ernst, E. (1999) *Lancet* **354**(9195), 2014-2016

138. Landes, N., Pfluger, P., Kluth, D., Birringer, M., Ruhl, R., Bol, G. F., Glatt, H., and Brigelius-Flohe, R. (2003) *Biochem Pharmacol* **65**(2), 269-273

139. Staudinger, J. L., Ding, X., and Lichti, K. (2006) *Expert Opin Drug Metab Toxicol* **2**(6), 847-857

140. Synold, T. W., Dussault, I., and Forman, B. M. (2001) *Nat Med* **7**(5), 584-590

141. Tabb, M. M., Kholodovych, V., Grun, F., Zhou, C., Welsh, W. J., and Blumberg, B. (2004) *Environ Health Perspect* **112**(2), 163-169

142. Takeshita, A., Taguchi, M., Koibuchi, N., and Ozawa, Y. (2002) *J Biol Chem* **277**(36), 32453-32458

143. Huang, H., Wang, H., Sinz, M., Zoeckler, M., Staudinger, J., Redinbo, M. R., Teotico, D. G., Locker, J., Kalpana, G. V., and Mani, S. (2007) *Oncogene* **26**(2), 258-268

144. Wang, H., Huang, H., Li, H., Teotico, D. G., Sinz, M., Baker, S. D., Staudinger, J., Kalpana, G., Redinbo, M. R., and Mani, S. (2007) *Clin Cancer Res* **13**(8), 2488-2495

145. Zhou, C., Poulton, E. J., Grun, F., Bammler, T. K., Blumberg, B., Thummel, K. E., and Eaton, D. L. (2007) *Mol Pharmacol* **71**(1), 220-229

146. Grant, D. M. (1991) *J Inherit Metab Dis* **14**(4), 421-430

147. Quattrochi, L. C., and Guzelian, P. S. (2001) *Drug Metab Dispos* **29**(5), 615-622

148. Huss, J. M., and Kasper, C. B. (1998) *J Biol Chem* **273**(26), 16155-16162

149. Lehmann, J. M., McKee, D. D., Watson, M. A., Willson, T. M., Moore, J. T., and Kliewer, S. A. (1998) *J Clin Invest* **102**(5), 1016-1023

150. Geick, A., Eichelbaum, M., and Burk, O. (2001) *J Biol Chem* **276**(18), 14581-14587

151. Goodwin, B., Moore, L. B., Stoltz, C. M., McKee, D. D., and Kliewer, S. A. (2001) *Mol Pharmacol* **60**(3), 427-431

152. Kast, H. R., Goodwin, B., Tarr, P. T., Jones, S. A., Anisfeld, A. M., Stoltz, C. M., Tontonoz, P., Kliewer, S., Willson, T. M., and Edwards, P. A. (2002) *J Biol Chem* **277**(4), 2908-2915

153. Gerbal-Chaloin, S., Daujat, M., Pascussi, J. M., Pichard-Garcia, L., Vilarem, M. J., and Maurel, P. (2002) *J Biol Chem* **277**(1), 209-217

154. Sueyoshi, T., and Negishi, M. (2001) *Annu Rev Pharmacol Toxicol* **41**, 123-143

155. Rosenfeld, J. M., Vargas, R., Jr., Xie, W., and Evans, R. M. (2003) *Mol Endocrinol* **17**(7), 1268-1282

156. Yang, J., and Yan, B. (2007) *Toxicol Sci* **95**(1), 13-22

157. Dunn, R. T., 2nd, Gleason, B. A., Hartley, D. P., and Klaassen, C. D. (1999) *J Pharmacol Exp Ther* **290**(1), 319-324

158. Falkner, K. C., Pinaire, J. A., Xiao, G. H., Geoghegan, T. E., and Prough, R. A. (2001) *Mol Pharmacol* **60**(3), 611-619

159. Hosokawa, M., Hattori, K., and Satoh, T. (1993) *Biochem Pharmacol* **45**(11), 2317-2322

160. Liu, L., and Klaassen, C. D. (1996) *Drug Metab Dispos* **24**(8), 854-858

161. Madhu, C., and Klaassen, C. D. (1991) *Toxicol Appl Pharmacol* **109**(2), 305-313

162. Runge-Morris, M., Wu, W., and Kocarek, T. A. (1999) *Mol Pharmacol* **56**(6), 1198-1206

163. Fromm, M. F., Kauffmann, H. M., Fritz, P., Burk, O., Kroemer, H. K., Warzok, R. W., Eichelbaum, M., Siegmund, W., and Schrenk, D. (2000) *Am J Pathol* **157**(5), 1575-1580

164. Handschin, C., and Meyer, U. A. (2003) *Pharmacol Rev* **55**(4), 649-673

165. Onate, S. A., Tsai, S. Y., Tsai, M. J., and O'Malley, B. W. (1995) *Science* **270**(5240), 1354-1357

166. Bhalla, S., Ozalp, C., Fang, S., Xiang, L., and Kemper, J. K. (2004) *J Biol Chem* **279**(43), 45139-45147

167. Brobst, D. E., Ding, X., Creech, K. L., Goodwin, B., Kelley, B., and Staudinger, J. L. (2004) *J Pharmacol Exp Ther* **310**(2), 528-535

168. Gonzalez, M. M., and Carlberg, C. (2002) *J Biol Chem* **277**(21), 18501-18509

169. Johnson, D. R., Li, C. W., Chen, L. Y., Ghosh, J. C., and Chen, J. D. (2006) *Mol Pharmacol* **69**(1), 99-108

170. Ourlin, J. C., Lasserre, F., Pineau, T., Fabre, J. M., Sa-Cunha, A., Maurel, P., Vilarem, M. J., and Pascussi, J. M. (2003) *Mol Endocrinol* **17**(9), 1693-1703

171. 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) *Cell* **85**(3), 403-414

172. Pazin, M. J., and Kadonaga, J. T. (1997) *Cell* **89**(3), 325-328

173. Spencer, T. E., Jenster, G., Burcin, M. M., Allis, C. D., Zhou, J., Mizzen, C. A., McKenna, N. J., Onate, S. A., Tsai, S. Y., Tsai, M. J., and O'Malley, B. W. (1997) *Nature* **389**(6647), 194-198

174. Yao, T. P., Ku, G., Zhou, N., Scully, R., and Livingston, D. M. (1996) *Proc Natl Acad Sci U S A* **93**(20), 10626-10631

175. Ding, X., and Staudinger, J. L. (2005) *J Pharmacol Exp Ther* **312**(2), 849-856

176. Mann, K. K., Padovani, A. M., Guo, Q., Colosimo, A. L., Lee, H. Y., Kurie, J. M., and Miller, W. H., Jr. (2005) *J Clin Invest* **115**(10), 2924-2933

177. Honkakoski, P., Zelko, I., Sueyoshi, T., and Negishi, M. (1998) *Mol Cell Biol* **18**(10), 5652-5658

178. Trottier, E., Belzil, A., Stoltz, C., and Anderson, A. (1995) *Gene* **158**(2), 263-268

179. Sueyoshi, T., Kawamoto, T., Zelko, I., Honkakoski, P., and Negishi, M. (1999) *J Biol Chem* **274**(10), 6043-6046

180. Tzameli, I., Pissios, P., Schuetz, E. G., and Moore, D. D. (2000) *Mol Cell Biol* **20**(9), 2951-2958

181. Wei, P., Zhang, J., Egan-Hafley, M., Liang, S., and Moore, D. D. (2000) *Nature* **407**(6806), 920-923

182. Maglich, J. M., Stoltz, C. M., Goodwin, B., Hawkins-Brown, D., Moore, J. T., and Kliewer, S. A. (2002) *Mol Pharmacol* **62**(3), 638-646

183. Araya, Z., and Wikvall, K. (1999) *Biochim Biophys Acta* **1438**(1), 47-54

184. Reichel, C., Gao, B., Van Montfoort, J., Cattori, V., Rahner, C., Hagenbuch, B., Stieger, B., Kamisako, T., and Meier, P. J. (1999) *Gastroenterology* **117**(3), 688-695

185. Goodwin, B., Jones, S. A., Price, R. R., Watson, M. A., McKee, D. D., Moore, L. B., Galardi, C., Wilson, J. G., Lewis, M. C., Roth, M. E., Maloney, P. R., Willson, T. M., and Kliewer, S. A. (2000) *Mol Cell* **6**(3), 517-526

186. Frank, C., Makkonen, H., Dunlop, T. W., Matilainen, M., Vaisanen, S., and Carlberg, C. (2005) *J Mol Biol* **346**(2), 505-519

187. Jung, D., Mangelsdorf, D. J., and Meyer, U. A. (2006) *J Biol Chem* **281**(28), 19081-19091

188. Jones, G., Strugnell, S. A., and DeLuca, H. F. (1998) *Physiol Rev* **78**(4), 1193-1231

189. Omdahl, J. L., Swamy, N., Serda, R., Annalora, A., Berne, M., and Rayb, R. (2004) *J Steroid Biochem Mol Biol* **89-90**(1-5), 159-162

190. Xu, Y., Iwanaga, K., Zhou, C., Cheesman, M. J., Farin, F., and Thummel, K. E. (2006) *Biochem Pharmacol* **72**(3), 385-392

191. Zhou, C., Assem, M., Tay, J. C., Watkins, P. B., Blumberg, B., Schuetz, E. G., and Thummel, K. E. (2006) *J Clin Invest* **116**(6), 1703-1712

192. Seo, Y. K., Chung, Y. T., Kim, S., Echchgadda, I., Song, C. S., and Chatterjee, B. (2007) *Gene* **386**(1-2), 218-223

193. Goldberg, A. D., Allis, C. D., and Bernstein, E. (2007) *Cell* **128**(4), 635-638

194. Hill, D. A. (2001) *Biochem Cell Biol* **79**(3), 317-324

195. Horn, P. J., and Peterson, C. L. (2002) *Science* **297**(5588), 1824-1827

196. Luger, K., Rechsteiner, T. J., Flaus, A. J., Waye, M. M., and Richmond, T. J. (1997) *J Mol Biol* **272**(3), 301-311

197. Morales, V., Giamarchi, C., Chailleux, C., Moro, F., Marsaud, V., Le Ricousse, S., and Richard-Foy, H. (2001) *Biochimie* **83**(11-12), 1029-1039

198. Orphanides, G., and Reinberg, D. (2000) *Nature* **407**(6803), 471-475

199. Urnov, F. D., and Wolffe, A. P. (2001) *Mol Endocrinol* **15**(1), 1-16

200. Kingston, R. E., and Narlikar, G. J. (1999) *Genes Dev* **13**(18), 2339-2352

201. Peterson, C. L., and Workman, J. L. (2000) *Curr Opin Genet Dev* **10**(2), 187-192

202. Arents, G., Burlingame, R. W., Wang, B. C., Love, W. E., and Moudrianakis, E. N. (1991) *Proc Natl Acad Sci U S A* **88**(22), 10148-10152

203. Luger, K., and Richmond, T. J. (1998) *Curr Opin Genet Dev* **8**(2), 140-146

204. Felsenfeld, G., and Groudine, M. (2003) *Nature* **421**(6921), 448-453

205. Jenuwein, T., and Allis, C. D. (2001) *Science* **293**(5532), 1074-1080

206. Turner, B. M. (2002) *Cell* **111**(3), 285-291

207. Somech, R., Izraeli, S., and A, J. S. (2004) *Cancer Treat Rev* **30**(5), 461-472

208. Nowak, S. J., and Corces, V. G. (2004) *Trends Genet* **20**(4), 214-220

209. Sterner, D. E., and Berger, S. L. (2000) *Microbiol Mol Biol Rev* **64**(2), 435-459

210. Zhang, Y., and Reinberg, D. (2001) *Genes Dev* **15**(18), 2343-2360

211. Shilatifard, A. (2006) *Annu Rev Biochem* **75**, 243-269

212. Nathan, D., Ingvarsdottir, K., Sterner, D. E., Bylebyl, G. R., Dokmanovic, M., Dorsey, J. A., Whelan, K. A., Krsmanovic, M., Lane, W. S., Meluh, P. B., Johnson, E. S., and Berger, S. L. (2006) *Genes Dev* **20**(8), 966-976

213. Hassa, P. O., Haenni, S. S., Elser, M., and Hottiger, M. O. (2006) *Microbiol Mol Biol Rev* **70**(3), 789-829

214. Cuthbert, G. L., Daujat, S., Snowden, A. W., Erdjument-Bromage, H., Hagiwara, T., Yamada, M., Schneider, R., Gregory, P. D., Tempst, P., Bannister, A. J., and Kouzarides, T. (2004) *Cell* **118**(5), 545-553

215. Wang, Y., Wysocka, J., Sayegh, J., Lee, Y. H., Perlin, J. R., Leonelli, L., Sonbuchner, L. S., McDonald, C. H., Cook, R. G., Dou, Y., Roeder, R. G., Clarke, S., Stallcup, M. R., Allis, C. D., and Coonrod, S. A. (2004) *Science* **306**(5694), 279-283

216. Nelson, C. J., Santos-Rosa, H., and Kouzarides, T. (2006) *Cell* **126**(5), 905-916

217. Allfrey, V. G., Faulkner, R., and Mirsky, A. E. (1964) *Proc Natl Acad Sci U S A* **51**, 786-794

218. Brownell, J. E., and Allis, C. D. (1996) *Curr Opin Genet Dev* **6**(2), 176-184

219. Kuo, M. H., and Allis, C. D. (1998) *Bioessays* **20**(8), 615-626

220. Roth, S. Y., Denu, J. M., and Allis, C. D. (2001) *Annu Rev Biochem* **70**, 81-120

221. Carrozza, M. J., Utley, R. T., Workman, J. L., and Cote, J. (2003) *Trends Genet* **19**(6), 321-329

222. Parthun, M. R., Widom, J., and Gottschling, D. E. (1996) *Cell* **87**(1), 85-94

223. Verreault, A., Kaufman, P. D., Kobayashi, R., and Stillman, B. (1998) *Curr Biol* **8**(2), 96-108

224. Verdin, E., Dequiedt, F., and Kasler, H. G. (2003) *Trends Genet* **19**(5), 286-293

225. Khan, A. U., and Hampsey, M. (2002) *Trends Genet* **18**(8), 387-389

226. van Leeuwen, F., Gafken, P. R., and Gottschling, D. E. (2002) *Cell* **109**(6), 745-756

227. Chen, Z., Zang, J., Whetstine, J., Hong, X., Davrazou, F., Kutateladze, T. G., Simpson, M., Mao, Q., Pan, C. H., Dai, S., Hagman, J., Hansen, K., Shi, Y., and Zhang, G. (2006) *Cell* **125**(4), 691-702

228. Shi, Y., Lan, F., Matson, C., Mulligan, P., Whetstine, J. R., Cole, P. A., Casero, R. A., and Shi, Y. (2004) *Cell* **119**(7), 941-953

229. Whetstine, J. R., Nottke, A., Lan, F., Huarte, M., Smolikov, S., Chen, Z., Spooner, E., Li, E., Zhang, G., Colaiacovo, M., and Shi, Y. (2006) *Cell* **125**(3), 467-481

230. Kouzarides, T. (2007) *Cell* **128**(4), 693-705

231. Wysocka, J., Swigut, T., Xiao, H., Milne, T. A., Kwon, S. Y., Landry, J., Kauer, M., Tackett, A. J., Chait, B. T., Badenhorst, P., Wu, C., and Allis, C. D. (2006) *Nature* **442**(7098), 86-90

232. Huang, J., Kent, J. R., Placek, B., Whelan, K. A., Hollow, C. M., Zeng, P. Y., Fraser, N. W., and Berger, S. L. (2006) *J Virol* **80**(12), 5740-5746

233. Pray-Grant, M. G., Daniel, J. A., Schieltz, D., Yates, J. R., 3rd, and Grant, P. A. (2005) *Nature* **433**(7024), 434-438

234. Sims, R. J., 3rd, Chen, C. F., Santos-Rosa, H., Kouzarides, T., Patel, S. S., and Reinberg, D. (2005) *J Biol Chem* **280**(51), 41789-41792

235. Fischle, W., Tseng, B. S., Dormann, H. L., Ueberheide, B. M., Garcia, B. A., Shabanowitz, J., Hunt, D. F., Funabiki, H., and Allis, C. D. (2005) *Nature* **438**(7071), 1116-1122

236. Clements, A., Poux, A. N., Lo, W. S., Pillus, L., Berger, S. L., and Marmorstein, R. (2003) *Mol Cell* **12**(2), 461-473

237. Briggs, S. D., Xiao, T., Sun, Z. W., Caldwell, J. A., Shabanowitz, J., Hunt, D. F., Allis, C. D., and Strahl, B. D. (2002) *Nature* **418**(6897), 498

238. Sun, Z. W., and Allis, C. D. (2002) *Nature* **418**(6893), 104-108

239. Lachner, M., O'Carroll, D., Rea, S., Mechtler, K., and Jenuwein, T. (2001) *Nature* **410**(6824), 116-120

240. Vakoc, C. R., Letting, D. L., Gheldof, N., Sawado, T., Bender, M. A., Groudine, M., Weiss, M. J., Dekker, J., and Blobel, G. A. (2005) *Mol Cell* **17**(3), 453-462

241. Driscoll, R., Hudson, A., and Jackson, S. P. (2007) *Science* **315**(5812), 649-652

242. Han, J., Zhou, H., Horazdovsky, B., Zhang, K., Xu, R. M., and Zhang, Z. (2007) *Science* **315**(5812), 653-655

243. Schneider, J., Bajwa, P., Johnson, F. C., Bhaumik, S. R., and Shilatifard, A. (2006) *J Biol Chem* **281**(49), 37270-37274

244. Chandy, M., Gutierrez, J. L., Prochasson, P., and Workman, J. L. (2006) *Eukaryot Cell* **5**(10), 1738-1747

245. Reinke, H., and Horz, W. (2003) *Mol Cell* **11**(6), 1599-1607

246. Zhao, J., Herrera-Diaz, J., and Gross, D. S. (2005) *Mol Cell Biol* **25**(20), 8985-8999

247. Vaquero, A., Scher, M. B., Lee, D. H., Sutton, A., Cheng, H. L., Alt, F. W., Serrano, L., Sternglanz, R., and Reinberg, D. (2006) *Genes Dev* **20**(10), 1256-1261

248. Shogren-Knaak, M., Ishii, H., Sun, J. M., Pazin, M. J., Davie, J. R., and Peterson, C. L. (2006) *Science* **311**(5762), 844-847

249. Kouzarides, T. (2002) *Curr Opin Genet Dev* **12**(2), 198-209

250. Strahl, B. D., Grant, P. A., Briggs, S. D., Sun, Z. W., Bone, J. R., Caldwell, J. A., Mollah, S., Cook, R. G., Shabanowitz, J., Hunt, D. F., and Allis, C. D. (2002) *Mol Cell Biol* **22**(5), 1298-1306

251. Wysocka, J., Swigut, T., Milne, T. A., Dou, Y., Zhang, X., Burlingame, A. L., Roeder, R. G., Brivanlou, A. H., and Allis, C. D. (2005) *Cell* **121**(6), 859-872

252. Pokholok, D. K., Harbison, C. T., Levine, S., Cole, M., Hannett, N. M., Lee, T. I., Bell, G. W., Walker, K., Rolfe, P. A., Herbolsheimer, E., Zeitlinger, J., Lewitter, F., Gifford, D. K., and Young, R. A. (2005) *Cell* **122**(4), 517-527

253. Carrozza, M. J., Li, B., Florens, L., Suganuma, T., Swanson, S. K., Lee, K. K., Shia, W. J., Anderson, S., Yates, J., Washburn, M. P., and Workman, J. L. (2005) *Cell* **123**(4), 581-592

254. Joshi, A. A., and Struhl, K. (2005) *Mol Cell* **20**(6), 971-978

255. Keogh, M. C., Kurdistani, S. K., Morris, S. A., Ahn, S. H., Podolny, V., Collins, S. R., Schuldiner, M., Chin, K., Punna, T., Thompson, N. J., Boone, C., Emili, A., Weissman, J. S., Hughes, T. R., Strahl, B. D., Grunstein, M., Greenblatt, J. F., Buratowski, S., and Krogan, N. J. (2005) *Cell* **123**(4), 593-605

256. Hiragami, K., and Festenstein, R. (2005) *Cell Mol Life Sci* **62**(23), 2711-2726

257. Smallwood, A., Esteve, P. O., Pradhan, S., and Carey, M. (2007) *Genes Dev* **21**(10), 1169-1178

258. Stallcup, M. R. (2001) *Oncogene* **20**(24), 3014-3020

259. Metivier, R., Penot, G., Hubner, M. R., Reid, G., Brand, H., Kos, M., and Gannon, F. (2003) *Cell* **115**(6), 751-763

260. Chang, B., Chen, Y., Zhao, Y., and Bruick, R. K. (2007) *Science* **318**(5849), 444-447

261. Cheung, P., Allis, C. D., and Sassone-Corsi, P. (2000) *Cell* **103**(2), 263-271

262. Johansen, K. M., and Johansen, J. (2006) *Chromosome Res* **14**(4), 393-404

263. Sassone-Corsi, P., Mizzen, C. A., Cheung, P., Crosio, C., Monaco, L., Jacquot, S., Hanauer, A., and Allis, C. D. (1999) *Science* **285**(5429), 886-891

264. Thomson, S., Clayton, A. L., Hazzalin, C. A., Rose, S., Barratt, M. J., and Mahadevan, L. C. (1999) *Embo J* **18**(17), 4779-4793

265. Dou, Y., and Gorovsky, M. A. (2002) *Proc Natl Acad Sci U S A* **99**(9), 6142-6146

266. Wang, H., Cao, R., Xia, L., Erdjument-Bromage, H., Borchers, C., Tempst, P., and Zhang, Y. (2001) *Mol Cell* **8**(6), 1207-1217

267. Gary, J. D., and Clarke, S. (1998) *Prog Nucleic Acid Res Mol Biol* **61**, 65-131

268. Boulanger, M. C., Miranda, T. B., Clarke, S., Di Fruscio, M., Suter, B., Lasko, P., and Richard, S. (2004) *Biochem J* **379**(Pt 2), 283-289

269. Hung, C. M., and Li, C. (2004) *Gene* **340**(2), 179-187

270. McBride, A. E., and Silver, P. A. (2001) *Cell* **106**(1), 5-8

271. Bedford, M. T., and Richard, S. (2005) *Mol Cell* **18**(3), 263-272

272. Bedford, M. T. (2007) *J Cell Sci* **120**(Pt 24), 4243-4246

273. Bedford, M. T., and Clarke, S. G. (2009) *Mol Cell* **33**(1), 1-13

274. Najbauer, J., Johnson, B. A., Young, A. L., and Aswad, D. W. (1993) *J Biol Chem* **268**(14), 10501-10509

275. Li, H., Park, S., Kilburn, B., Jelinek, M. A., Henschen-Edman, A., Aswad, D. W., Stallcup, M. R., and Laird-Offringa, I. A. (2002) *J Biol Chem* **277**(47), 44623-44630

276. Miranda, T. B., Miranda, M., Frankel, A., and Clarke, S. (2004) *J Biol Chem* **279**(22), 22902-22907

277. Chen, D., Ma, H., Hong, H., Koh, S. S., Huang, S. M., Schurter, B. T., Aswad, D. W., and Stallcup, M. R. (1999) *Science* **284**(5423), 2174-2177

278. Stallcup, M. R., Chen, D., Koh, S. S., Ma, H., Lee, Y. H., Li, H., Schurter, B. T., and Aswad, D. W. (2000) *Biochem Soc Trans* **28**(4), 415-418

279. Koh, S. S., Chen, D., Lee, Y. H., and Stallcup, M. R. (2001) *J Biol Chem* **276**(2), 1089-1098

280. Meyer, R., Wolf, S. S., and Obendorf, M. (2007) *J Steroid Biochem Mol Biol* **107**(1-2), 1-14

281. Ganesh, L., Yoshimoto, T., Moorthy, N. C., Akahata, W., Boehm, M., Nabel, E. G., and Nabel, G. J. (2006) *Mol Cell Biol* **26**(10), 3864-3874

282. Yadav, N., Cheng, D., Richard, S., Morel, M., Iyer, V. R., Aldaz, C. M., and Bedford, M. T. (2008) *EMBO Rep* **9**(2), 193-198

283. Zhao, X., Jankovic, V., Gural, A., Huang, G., Pardanani, A., Menendez, S., Zhang, J., Dunne, R., Xiao, A., Erdjument-Bromage, H., Allis, C. D., Tempst, P., and Nimer, S. D. (2008) *Genes Dev* **22**(5), 640-653

284. Lee, Y. H., Koh, S. S., Zhang, X., Cheng, X., and Stallcup, M. R. (2002) *Mol Cell Biol* **22**(11), 3621-3632

285. An, W., Kim, J., and Roeder, R. G. (2004) *Cell* **117**(6), 735-748

286. Huang, S., Litt, M., and Felsenfeld, G. (2005) *Genes Dev* **19**(16), 1885-1893

287. Daujat, S., Bauer, U. M., Shah, V., Turner, B., Berger, S., and Kouzarides, T. (2002) *Curr Biol* **12**(24), 2090-2097

288. Xu, W., Cho, H., Kadam, S., Banayo, E. M., Anderson, S., Yates, J. R., 3rd, Emerson, B. M., and Evans, R. M. (2004) *Genes Dev* **18**(2), 144-156

289. Lee, Y. H., Campbell, H. D., and Stallcup, M. R. (2004) *Mol Cell Biol* **24**(5), 2103-2117

290. Vo, N., Fjeld, C., and Goodman, R. H. (2001) *Mol Cell Biol* **21**(18), 6181-6188

291. Mowen, K. A., Tang, J., Zhu, W., Schurter, B. T., Shuai, K., Herschman, H. R., and David, M. (2001) *Cell* **104**(5), 731-741

292. Mostaqul Huq, M. D., Gupta, P., Tsai, N. P., White, R., Parker, M. G., and Wei, L. N. (2006) *Embo J* **25**(21), 5094-5104

293. Man, Y. G., Martinez, A., Avis, I. M., Hong, S. H., Cuttitta, F., Venzon, D. J., and Mulshine, J. L. (2000) *Am J Respir Cell Mol Biol* **23**(5), 636-645

294. Ancelin, K., Lange, U. C., Hajkova, P., Schneider, R., Bannister, A. J., Kouzarides, T., and Surani, M. A. (2006) *Nat Cell Biol* **8**(6), 623-630

295. Fabbrizio, E., El Messaoudi, S., Polanowska, J., Paul, C., Cook, J. R., Lee, J. H., Negre, V., Rousset, M., Pestka, S., Le Cam, A., and Sardet, C. (2002) *EMBO Rep* **3**(7), 641-645

296. Hou, Z., Peng, H., Ayyanathan, K., Yan, K. P., Langer, E. M., Longmore, G. D., and Rauscher, F. J., 3rd. (2008) *Mol Cell Biol* **28**(10), 3198-3207

297. Pal, S., Yun, R., Datta, A., Lacomis, L., Erdjument-Bromage, H., Kumar, J., Tempst, P., and Sif, S. (2003) *Mol Cell Biol* **23**(21), 7475-7487

298. Pal, S., Vishwanath, S. N., Erdjument-Bromage, H., Tempst, P., and Sif, S. (2004) *Mol Cell Biol* **24**(21), 9630-9645

299. Guccione, E., Bassi, C., Casadio, F., Martinato, F., Cesaroni, M., Schuchlautz, H., Luscher, B., and Amati, B. (2007) *Nature* **449**(7164), 933-937

300. Hyllus, D., Stein, C., Schnabel, K., Schiltz, E., Imhof, A., Dou, Y., Hsieh, J., and Bauer, U. M. (2007) *Genes Dev* **21**(24), 3369-3380

301. Iberg, A. N., Espejo, A., Cheng, D., Kim, D., Michaud-Levesque, J., Richard, S., and Bedford, M. T. (2008) *J Biol Chem* **283**(6), 3006-3010

302. Jelinic, P., Stehle, J. C., and Shaw, P. (2006) *PLoS Biol* **4**(11), e355

303. Kwak, Y. T., Guo, J., Prajapati, S., Park, K. J., Surabhi, R. M., Miller, B., Gehrig, P., and Gaynor, R. B. (2003) *Mol Cell* **11**(4), 1055-1066

304. Chevillard-Briet, M., Trouche, D., and Vandel, L. (2002) *Embo J* **21**(20), 5457-5466

305. Goodwin, G. H., Rabbani, A., Nicolas, P. H., and Johns, E. W. (1977) *FEBS Lett* **80**(2), 413-416

306. Sgarra, R., Diana, F., Bellarosa, C., Dekleva, V., Rustighi, A., Toller, M., Manfioletti, G., and Giancotti, V. (2003) *Biochemistry* **42**(12), 3575-3585

307. Lin, W. J., Gary, J. D., Yang, M. C., Clarke, S., and Herschman, H. R. (1996) *J Biol Chem* **271**(25), 15034-15044

308. Robin-Lespinasse, Y., Sentis, S., Kolytcheff, C., Rostan, M. C., Corbo, L., and Le Romancer, M. (2007) *J Cell Sci* **120**(Pt 4), 638-647

309. Singh, V., Miranda, T. B., Jiang, W., Frankel, A., Roemer, M. E., Robb, V. A., Gutmann, D. H., Herschman, H. R., Clarke, S., and Newsham, I. F. (2004) *Oncogene* **23**(47), 7761-7771

310. Higashimoto, K., Kuhn, P., Desai, D., Cheng, X., and Xu, W. (2007) *Proc Natl Acad Sci U S A* **104**(30), 12318-12323

311. Sayegh, J., Webb, K., Cheng, D., Bedford, M. T., and Clarke, S. G. (2007) *J Biol Chem* **282**(50), 36444-36453

312. Rathert, P., Zhang, X., Freund, C., Cheng, X., and Jeltsch, A. (2008) *Chem Biol* **15**(1), 5-11

313. Kliewer, S. A., and Willson, T. M. (2002) *J Lipid Res* **43**(3), 359-364

314. Kliewer, S. A., Goodwin, B., and Willson, T. M. (2002) *Endocr Rev* **23**(5), 687-702

315. Qi, C., Chang, J., Zhu, Y., Yeldandi, A. V., Rao, S. M., and Zhu, Y. J. (2002) *J Biol Chem* **277**(32), 28624-28630

316. Rizzo, G., Renga, B., Antonelli, E., Passeri, D., Pellicciari, R., and Fiorucci, S. (2005) *Mol Pharmacol* **68**(2), 551-558

317. Tian, Y. (2009) *Biochem Pharmacol* **77**(4), 670-680

318. Gu, X., Ke, S., Liu, D., Sheng, T., Thomas, P. E., Rabson, A. B., Gallo, M. A., Xie, W., and Tian, Y. (2006) *J Biol Chem* **281**(26), 17882-17889

319. Cote, J., Boisvert, F. M., Boulanger, M. C., Bedford, M. T., and Richard, S. (2003) *Mol Biol Cell* **14**(1), 274-287

320. Tang, J., Frankel, A., Cook, R. J., Kim, S., Paik, W. K., Williams, K. R., Clarke, S., and Herschman, H. R. (2000) *J Biol Chem* **275**(11), 7723-7730

321. Nishioka, K., and Reinberg, D. (2003) *Methods* **31**(1), 49-58

322. Pawlak, M. R., Scherer, C. A., Chen, J., Roshon, M. J., and Ruley, H. E. (2000) *Mol Cell Biol* **20**(13), 4859-4869

323. Balint, B. L., Szanto, A., Madi, A., Bauer, U. M., Gabor, P., Benko, S., Puskas, L. G., Davies, P. J., and Nagy, L. (2005) *Mol Cell Biol* **25**(13), 5648-5663

324. Friend, C., Scher, W., Holland, J. G., and Sato, T. (1971) *Proc Natl Acad Sci U S A* **68**(2), 378-382

325. Marks, P. A., and Breslow, R. (2007) *Nat Biotechnol* **25**(1), 84-90

326. Yen, A. (1985) *Exp Cell Res* **156**(1), 198-212

VITA

Name: Ying Xie

Address: Mailstop 4466, VTPP, TAMU
College Station, TX 77843

Email Address: yxie@cvm.tamu.edu

Education: B.S., Biological Science, Peking University, China, 2003
Ph.D., Toxicology,
Department of Veterinary Pharmacology and Physiology
Texas A&M University**,** College Station, TX, May, 2010