Interaction of PIMT with Transcriptional Coactivators CBP, p300, and PBP Differential Role in Transcriptional Regulation*

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PIMT (PRIP-interacting protein with methyltransferase domain), an RNA-binding protein with a methyltransferase domain capable of binding S-adenosylmethionine, has been shown previously to interact with nuclear receptor coactivator PRIP (peroxisome proliferator-activated receptor (PPAR)-interacting protein) and enhance its coactivator function. We now report that PIMT strongly interacts with transcriptional coactivators, CBP, p300, and PBP but not with SRC-1 and PGC-1α under in vitro and in vivo conditions. The PIMT binding sites on CBP and p300 are located in the cysteine-histidine-rich C/H1 and C/H3 domains, and the PIMT binding site on PBP is in the region encompassing amino acids 1101–1360. The N-terminal of PIMT (residues 1–509) containing the RNA binding domain interacts with both C/H1 and C/H3 domains of CBP and p300 and with the C-terminal portion of PBP that encompasses amino acids 1371–1560. The C-terminal of PIMT (residues 611–852), which binds S-adenosyl-L-methionine, interacts respectively with the C/H3 domain of CBP/p300 and with a region encompassing amino acids 1101–1370 of PBP. Immunoprecipitation data showed that PIMT forms a complex in vivo with CBP, p300, PBP, and PRIP. PIMT appeared to co-localize in the nucleus with CBP, p300, and PBP. PIMT enhanced PBP-mediated transcriptional activity of the PPARγ, as it did for PRIP, indicating synergism between PIMT and PBP. In contrast, PIMT functioned as a repressor of CBP/p300-mediated transactivation of PPARγ. Based on these observations, we suggest that PIMT bridges the CBP/p300-anchored coactivator complex with the PBP-anchored coactivator complex but differentially modulates coactivator function such that inhibition of the CBP/p300 effect may be designed to enhance the activity of PBP and PRIP.

The nuclear receptor superfamily consists of several ligand-regulated transcription factors that include the steroid and thyroid hormone receptors, vitamin D$_3$ receptor, retinoic acid receptors, and the peroxisome proliferator-activated receptors (PPARs), 1 among others (1–3). Liganded nuclear receptors participate in diverse biological processes by controlling gene expression patterns in a cell-specific manner. This is accomplished by interacting with specific response elements (DNA sequence) located in the promoter regions of target genes, by recruiting transcriptional cofactors that function in nucleosome remodeling, and by recruiting RNA polymerase II holoenzyme to initiate transcription (1, 2, 4, 5). In recent years many nuclear receptor-interacting cofactors termed coactivators have been identified (4, 5). These coactivators, which enhance transcriptional activation of nuclear receptors, include the p160/SRC-1 (steroid receptor coactivator-1) family with three members (SRC-1, TIF2/GRIP1/SRC2, and pCIP/ACTR/AIB1/RAC3/ TRAM1/SRC-3) (6–12), CREB-binding protein (CBP) (13), adenosine A1A-binding protein p300 (14), PPAR-binding protein (PPP) (TRAP220/DRIP205) (15, 16), PPARγ coactivators PGC-1α and PGC-1β (17, 18), and the general coactivator PRIP/ASC-2/RAP250/TRBP/NRC (18–22), among others (23). They all contain one or more conserved LXXLL (where L is leucine and X is any amino acid) signature motifs, which have been found to be necessary and sufficient for ligand-dependent interactions with the activation function-2 (AF-2) domain present in the C-terminal nuclear receptor ligand binding domain (12, 24). There is increasing appreciation that while some coactivators, such as p160/SRC-1 family members, interact predominantly with nuclear receptors to enhance gene-specific transcription (2, 5), others (for example CBP/p300 and PBP) appear to interact with nuclear receptors and many other transcription factors (25–27). Nuclear receptors and their coactivators regulate the transcriptional activity of target genes that play central roles in key biological phenomena such as early development, cell proliferation, differentiation, apoptosis, metabolic homeostasis, and cancer (25).

The p160 family of nuclear receptor coactivators and CBP/p300 possess intrinsic histone acetyltransferase (HAT) activity in addition to their role of enhancing transcriptional activation in transient transfection assays (5, 10, 28, 29). These coactivators also recruit other proteins with HAT activity to form a

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1 The abbreviations used are: PPAR, peroxisome proliferator-activated receptor; PPRE, peroxisome proliferator-activated response element; SRC-1, steroid receptor coactivator-1; PBP, PPAR-binding protein; CREB, cAMP response element-binding protein; CBP, CREB-binding protein; TRAP, thyroid hormone receptor-associated protein(s); DRIP, vitamin D receptor-interacting protein(s); ARC, activator-recruited cofactor; CARM1, coactivator-associated arginine methyltransferase-1; PRMT1, protein arginine methyltransferase-1; PRIP, PPAR-interacting protein; PIMT, PRIP-interacting protein with methyltransferase domain; RXR, retinoid-X receptor for 9-cis-retinoic acid; GST, glutathione S-transferase; aa, amino acid(s); HAT, histone acetyltransferase; Ad, adenosine; C/H, cysteine-histidine-rich domain; HEK, human embryonic kidney.
multiprotein complex anchored by CBP/p300. This complex regulates transcription through remodeling chromatin by acetylating histones (30). Recently, coactivator-associated enhancement of transcription has also been shown to involve the recruitment of coactivator-associated proteins such as coactivator-associated arginine methyltransferase 1 (CARM1), a member of the S-adenosyl-l-methionine-dependent, protein arginine methyltransferase (PRMT) family (31). CARM1 catalyzes the methylation of arginine residues particularly in histone 3 (31). The PRMT family consists of at least five members (PRMT1, PRMT2, PRMT3, PRMT4/CARM1, and PRMT5) (32) of which CARM1/PRMT4 is known to enhance the function of the p160 family of coactivators by increasing nuclear receptor-dependent gene transcription. CARM1/PRMT4 does this by binding to and methylating the KIX domain of CBP/p300 in addition to its initially described role in histone 3 methylation (33). The KIX domain-methylated CBP/p300 fails to activate CREB-dependent genes. Therefore, the CBP/p300 is able to direct nuclear receptor-dependent gene transcription (33). From these observations it appears that histone acetylation, histone methylation, and selective coactivator methylation (namely CBP/p300 methylation) constitute novel regulatory mechanisms that involve the CBP/p300-anchored coactivator complex in nuclear receptor-mediated gene transcription.

The next step in the multistep transcriptional activation process involves participation of the TRAP/DRIP/ARC mediator complex anchored by the coactivator PBP (4, 5, 26). While CBP/p300 and the p160 family of cofactors that form the initial multiprotein complex function by exhibiting HAT and arginine methyltransferase activities, there is limited functional information about the coactivator PBP and other proteins that form the TRAP/DRIP/ARC complex. This complex facilitates interaction with RNA polymerase II complexes of the basal transcriptional machinery, but how this is accomplished remains unclear (4, 5, 16, 20, 34, 35). PBP lacks HAT activity, and it is uncertain if the other members of the TRAP/DRIP/ARC complex (including CBP/p300 of the TRAP/DRIP/ARC complex function by exhibiting HAT and arginine methyltransferase-histone methyltransferase complex of CBP/p300 and p160 coactivators and the downstream TRAP/DRIP/ARC complex. Disruption of the PRMT family of coactivators affects the function of many nuclear receptors and possibly other transcription factors (27, 36–40). The recently identified coactivator designated PRIP/ASC2/RAP250/NRC/TRBP has been shown to interact with several nuclear receptors and also with CBP/p300 and TRAP130 of the TRAP/DRIP/ARC complex (18–22). Thus, PRIP appears to serve as a bridge between the initial histone acetyltransferase-histone methyltransferase complex of CBP/p300 and p160 coactivators and the downstream TRAP/DRIP/ARC complex. Disruption of this PRIP gene in the mouse leads to embryonic lethality around E13.5 days, implying that PRIP interacts with CBP/p300 and possibly other transcription factors (27, 36–40). The recently identified coactivator designated PRIP/ASC2/RAP250/NRC/TRBP has been shown to interact with several nuclear receptors and also with CBP/p300 and TRAP130 of the TRAP/DRIP/ARC complex (18–22). Thus, PRIP appears to serve as a bridge between the initial histone acetyltransferase-histone methyltransferase complex of CBP/p300 and p160 coactivators and the downstream TRAP/DRIP/ARC complex. Disruption of this PRIP gene in the mouse leads to embryonic lethality around E13.5 days, implying that PRIP interacts with CBP/p300 and possibly other transcription factors (27, 36–40). The recently identified coactivator designated PRIP/ASC2/RAP250/NRC/TRBP has been shown to interact with several nuclear receptors and also with CBP/p300 and TRAP130 of the TRAP/DRIP/ARC complex (18–22). Thus, PRIP appears to serve as a bridge between the initial histone acetyltransferase-histone methyltransferase complex of CBP/p300 and p160 coactivators and the downstream TRAP/DRIP/ARC complex. Disruption of this PRIP gene in the mouse leads to embryonic lethality around E13.5 days, implying that PRIP interacts with CBP/p300 and possibly other transcription factors (27, 36–40). The recently identified coactivator designated PRIP/ASC2/RAP250/NRC/TRBP has been shown to interact with several nuclear receptors and also with CBP/p300 and TRAP130 of the TRAP/DRIP/ARC complex (18–22). Thus, PRIP appears to serve as a bridge between the initial histone acetyltransferase-histone methyltransferase complex of CBP/p300 and p160 coactivators and the downstream TRAP/DRIP/ARC complex. Disruption of this PRIP gene in the mouse leads to embryonic lethality around E13.5 days, implying that PRIP interacts with CBP/p300 and possibly other transcription factors (27, 36–40). The recently identified coactivator designated PRIP/ASC2/RAP250/NRC/TRBP has been shown to interact with several nuclear receptors and also with CBP/p300 and TRAP130 of the TRAP/DRIP/ARC complex (18–22). Thus, PRIP appears to serve as a bridge between the initial histone acetyltransferase-histone methyltransferase complex of CBP/p300 and p160 coactivators and the downstream TRAP/DRIP/ARC complex. Disruption of this PRIP gene in the mouse leads to embryonic lethality around E13.5 days, implying that PRIP interacts with CBP/p300 and possibly other transcription factors. We found that PRIP interacts with CBP/p300 and PBP but not with SRC-1 and PGCo, suggesting that PRIP functions as a link between the two major multiprotein complexes an-

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2 C. Qi and J. K. Reddy, unpublished observations.

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**EXPERIMENTAL PROCEDURES**

**Plasmid Constructions**—pCDNA3.1-PRIP, pCDNA3.1-PI-MT (aa 1–369), pGEX-PI-MT (aa 577–852), pGEX-PI-MT (aa 326–852), pCMV-3XFLAG-PI-MT, pCMX-PBP, pCDNA3.1-PRIP, pCDNA3.1-PRIP, pCMV-p300, 3XPPRE-Luc, and pCMV-PAR-1 have been described (41). pCDNA3.1-1-PGClα was a generous gift from Dr. Bruce M. Djeugetman, pCDNA3.1-1-HPA, was constructed by subcloning the HindIII-InhoI fragment containing the entire coding region of CBP from pRc/RSV-CBP. HA into pCDNA3.1. Sequences encoding peptide fragments of entire CBP, p300 and PBP fused to glutathione S-transferase (GST) (hereafter GST-CBP, GST-p300, and GST-PBP) were generated by subcloning respective PCR fragments amplified from the appropriate plasmids into the correct reading frame of pGEX4T1 (Amersham Biosciences). GST-PMT (aa 1–334), pCDNA3.1-PI-MT (aa 370–610), and pCDNA3.1 PMT (aa 611–852) were generated by subcloning PCR fragments into the pGEX and pCDNA3.1, respectively. Sequences of all clones were verified by sequencing.

**Construction of Adenovirus PI-MT**—To construct recombinant adenoviral PI-MT, PI-MT cDNA was cloned by PCR from plasmid pCMV-PI-MT (41) and inserted into the BamHI/XhoI site of pFastbac HTc (Invitrogen). The entire coding region of PI-MT with the hexahistidine affinity tag was cut with RuvC/XhoI and transferred to the SalI site of pShuttle vector (Quantum Biotechnologies, Inc.) by blunting the ends. The recombinant Ad/PI-MT was generated as described previously (42). The adenoviral construct of Ad/LacZ was the generous gift of Dr. W. El-Deiry (University of Pennsylvania, Philadelphia) and has been described previously (43).

**GST Pull-down Assays**—GST fusions or GST alone was expressed in Escherichia coli BL21 (DE3) bound to glutathione-Sepharose 4B beads (Amersham Biosciences) and incubated with [35S]methionine-labeled coactivator/coactivator-binding protein expressed by in vitro transcription/translation using the T7-Coupled transcription-translation system (Promega). Briefly, the binding assays were carried out by incubating 10 μl of [35S]methionine-labeled coactivator/coactivator-binding protein for 2 h at 4°C with the immobilized GST fusion protein in GST binding buffer (180 mM KCl, 20 mM Tris-HCl, pH 7.9, 1 mM EDTA, 0.05% Nonidet P-40, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride). Beads were washed four times with 1 ml of binding buffer containing 0.1% Nonidet P-40, and bound protein was eluted by boiling for 2 min in 20 μl of SDS sample buffer, was analyzed by SDS-PAGE, and was subjected to autoradiography.

**Immunoprecipitation and Western Blotting**—COS-7 cells were cultured in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum and transfected with pCMV-PI-MT-FLAG plasmid encoding FLAG-tagged PI-MT along with one of the coactivator expression plasmids, transfected with h cells were harvested and lysed at 2°C for overnight in lysis buffer (100 mM Tris-HCl, pH 8.0, 250 mM NaCl, 1% Nonidet P-40, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride). Beads were washed four times with 1 ml of binding buffer containing 0.1% Nonidet P-40, and bound protein was eluted by boiling for 2 min in 20 μl of SDS sample buffer, was analyzed by SDS-PAGE, and was subjected to autoradiography.
**RESULTS**

**PIMT Interacts with CBP, p300, PBP, and PRIP but Not with PGC1 and SRC-1**—Previously, we have shown that PIMT interacts with PRIP (41). To determine whether PIMT interacts with other coactivators we used bacterially generated GST fusion of truncated PIMT containing the RNA methyltransferase domain encompassing aa 577–852 (hereafter, PIMTC) (Fig. 1A) and tested its direct *in vitro* interaction with *in vitro* translated CBP, p300, PBP, PGC-1, and SRC-1. As shown in Fig. 1B, CBP, PBP, PRIP, and p300 interacted with immobilized GST-PIMTC, but PGC-1 and SRC-1 failed do so.

Having confirmed that PGC-1α and SRC-1 failed to interact with truncated PIMT (PIMTC), we then tested the interaction of GST-PIMTA (aa 1–334) and GST-PIMTB (aa 326–852) with *in vitro* translated [*35S*]methionine-labeled full-length SRC-1 and PGC-1α (Fig. 1C). We failed to detect any binding of SRC-1 and PGC-1α with these PIMT fragments. We also used full-length *in vitro* translated PIMT and investigated its *in vitro* interaction with various SRC-1- and PGC-1α-truncated proteins expressed as GST fusions. In these assays full-length PIMT did not interact with these two coactivators (data not shown).

**Mapping of the CBP and PIMT Domains Required for Interaction**—In order to define the interacting domain(s) of CBP on PIMT, we prepared GST fusion proteins containing various peptides of CBP and determined the ability of each of these to interact with [*35S*]methionine-labeled *in vitro* translated full-length PIMT. GST and GST-CBP fusion proteins (shown schematically in Fig. 2A) were bacterially expressed, purified, coupled to glutathione-Sepharose beads, and used for *in vitro* binding assays. CBP2 peptide (aa 301–600) containing the cysteine-histidine-rich 1 (C/H1) region and CBP7 peptide (aa 1801–2100) containing the cysteine-histidine-rich 3 (C/H3) region interacted significantly with full-length PIMT (Fig. 2B). The extent of interaction of PIMT with CBP2 peptide is decreased when a pull-down assay was done at the higher salt concentration of 250 mM KCl, indicating weak interaction of PIMT with peptide containing the C/H1 domain compared with that of peptide containing the C/H3 domain (data not shown). Using further truncated CBP2 and CBP7 fragments, we narrowed down the PIMT binding sites on CBP to two regions encompassing amino acids 301–400 (Fig. 2B, CBP2A) and 1801–1900 (Fig. 2B, CBP7A), respectively.

We then determined the domain(s) of PIMT that are important for CBP interaction. Three different PIMT peptides (namely PIMTN (aa 1–369), PIMTM (aa 370–610), and PIMTC (aa 611–852)) were translated *in vitro* in the presence of...
[35S]methionine, and equal portions of these peptides were used to assess their binding to GST alone, GST-CBP2A, and GST-CBP7A in the in vitro pull-down assays (Fig. 2). Both PIMTN (aa 1–369) and PIMTC (aa 611–852) interacted with CBP7A (aa 1801–1900), whereas only PIMTN interacted with CBP2A (aa 301–400) (Fig. 2C), indicating two independent domains of interaction of PIMTN on CBP. PIMTM did not bind these GST-CBP fusion peptides. We can conclude that PIMT has two independent binding sites on CBP, one in the region of aa 301–400 and the other in the region of aa 1801–1900. These CBP regions also interact with adenoviral E1A oncoprotein (44).

**FIG. 2.** Identification of CBP- and PIMT-interacting domains. A, physical map of CBP depicting various domains: NR, nuclear hormone receptor domain; C/H1, cysteine-histidine-rich domain 1; C/H3, cysteine-histidine-rich domain 3; K/X, phospho-CREB binding domain. PIMT interacts with the C/H1 and C/H3 regions of CBP as shown in B and C below. B, different fragments of GST-CBP were immobilized on GST-Sepharose and incubated with [35S]methionine-labeled in vitro translated full-length PIMT. Full-length PIMT binds to peptides CBP2 (aa 301–600) and CBP7 (aa 1801–2100). The binding sites of PIMT on CBP have been further narrowed down to the regions encompassing the C/H1 (aa 301–400) and C/H3 (aa 1801–1900) domains. C, identification of PIMT domains that interact with CBP. [35S]methionine-labeled in vitro translated PIMTN, PIMTM, and PIMTC fragments were incubated with GST-CBP2A and GST-CBP7A. PIMTN binding sites on CBP appear to be located in regions of aa 301–400 and aa 1801–1900, whereas the PIMTC binding domain on CBP is located in the region encompassing aa 1801–1900. PIMTM did not bind with these GST-CBP fragments.

**FIG. 3.** Identification of p300 and PIMT domains that interact with each other. A, physical map of p300 showing NR, C/H1, C/H3, K/X, and HAT domains. PIMT interacts with the C/H1 and C/H3 regions of p300 based on the results shown in B and C below. B, schematic diagram of GST-p300 fusion peptides used in pull-down assays, and demonstration that full-length [35S]methionine-labeled PIMT interacts with p300/2A (aa 301–400) and p300/5C (aa 1701–1800). C, identification of PIMT domains that interact with p300. [35S]methionine-labeled in vitro translated PIMTN, PIMTM, and PIMTC fragments were incubated with GST-p300/2A and GST-p300/5C. PIMTN binding sites on p300 appear to be located in regions of aa 301–400 and aa 1801–1900, whereas the PIMTC binding domain on p300 is located in the region encompassing aa 1801–1900. PIMTM did not bind with these GST-CBP fragments.
encompassing aa 301–920 and aa 1701–1800 (Fig. 3B). The results of the binding of PIMT peptides (PIMTN, PIMTM, and PIMTC) to p300 clearly established that the PIMT N and C domains interact(s) with p300 (Fig. 3C).

Mapping of the PBP and PIMT Domains Required for Interaction—In vitro GST pull-down analyses were performed with recombinant GST fusion proteins of PBP and [35S]methionine-labeled in vitro translated PIMT proteins. GST-PBP fragments were generated to map the regions required for interaction with PIMT (Fig. 4, A and B). Three overlapping fragments in the C-terminal of PBP (PBP5, PBP6, and PBP7 covering aa 740–1130, 980–1370, and 1360–1560, respectively) interacted with various fragments of PBP. PIMT binds with three overlapping GST-PBP fragments, PBP5 (aa 740–1130), PBP6 (aa 980–1370), and PBP7 (aa 1371–1560). C, identification of PIMT domains that interact with PBP. [35S]methionine-labeled PIMTN (aa 1–369), PIMTM (aa 370–610), and PIMTC (aa 611–852) were allowed to interact with various GST-PBP fragments encompassing PBP region aa 981–1560. PIMTN binds with the PBP11 (aa 1371–1560), and PIMTC binds with PBP9 (aa 1101–1370). PIMTM did not bind with any GST-PBP fragment.

FIG. 4. Identification of PBP- and PIMT-interacting domains. A, map of PBP depicting the LXXL and PIMT-interacting regions. B, GST pull-down assays using various fragments of PBP. [35S]methionine-labeled full-length PIMT generated by in vitro translation was incubated with glutathione-Sepharose beads bound with purified E. coli expressed GST or different fragments of PBP. PIMT binds with three overlapping GST-PBP fragments, PBP5 (aa 740–1130), PBP6 (aa 980–1370), and PBP7 (aa 1371–1560). C, identification of PIMT domains that interact with PBP. [35S]methionine-labeled PIMTN (aa 1–369), PIMTM (aa 370–610), and PIMTC (aa 611–852) were allowed to interact with various GST-PBP fragments encompassing PBP region aa 981–1560. PIMTN binds with the PBP11 (aa 1371–1560), and PIMTC binds with PBP9 (aa 1101–1370). PIMTM did not bind with any GST-PBP fragment.

Interaction of PIMT with CBP, p300, PBP, and PRIP In Vivo (PIMT Complex)—Given that PIMT interacts with the nuclear receptor coactivators CBP, p300, and PBP in vitro, we wanted to determine whether PIMT interacts with CBP, p300, and PBP in the context of intact cells. We used two different approaches. First, a vector encoding human PIMT with the C-terminal FLAG epitope was cotransfected separately along with one of the coactivators (namely CBP, p300, PBP, and PRIP) (used as positive control) into COS-1 cells derived from African Green Monkey Kidney (American Type Culture Collection, CRL1651). The potential complex between PIMT and each coactivator was immunoprecipitated separately using respective antibodies (i.e. anti-CBP, anti-p300, anti-PBP, and anti-PRIP), and the products were analyzed by immunoblot using anti–FLAG to demonstrate the presence of PIMT in the precipitates (Fig. 5A). The results showed that all antibodies precipitated PIMT, demonstrating that PIMT interacts in vivo with these nuclear receptor coactivators (Fig. 5A) and suggesting the existence of a PIMT-coactivator complex in vivo. Second, to pull down such a complex, we infected HEK293 cells with Ad/PIMT or Ad/LacZ and immunoprecipitated recombinant PIMT using anti-His antibodies. The immunoprecipitate obtained in this fashion contained CBP, p300, PBP, PRIP, as well as PIMT (Fig. 5B), indicating the existence of a complex of at least five proteins anchored by or containing PIMT as a component.

FIG. 5. PIMT interacts with CBP, p300, PRIP and PBP In Vivo. A, cell lysates were prepared from COS-7 cells transfected with pCDNA3.1-FLAG-PIMT and one of the coactivator expression plasmids separately and immunoprecipitated with anti-CBP, anti-p300, anti-PRIP, or anti-PBP antibodies. Immunoprecipitates were subjected to SDS-PAGE and immunoblotted with anti-FLAG epitope-tagged protein. PIMT is coimmunoprecipitated by anti-CBP (lane 3), anti-p300 (lane 4), anti-PRIP (lane 5) and anti-PBP (lane 6) but not by preimmune serum (lane 2). Lane 1 represents cell lysate (1/20 input). B, HEK293 cells infected with Ad/LacZ or Ad/PIMT were lysed and immunoprecipitated with anti-His tag. The immunoprecipitates when subjected to immunoblotting with anti-CBP, anti-p300, anti-PRIP, or anti-FLAG (for PIMT) reveal that PIMT exists as a complex with these coactivators.

Interaction of PIMT with CBP, p300, PBP, and PRIP In Vivo—In vivo and in vitro. Previously, we demonstrated by immunofluorescence microscopy that PIMT and PRIP colocalize within the nucleus. To evaluate whether PIMT colocalizes with its newly identified binding partners, a plasmid containing three FLAG epitopes linked to the C-terminal portion of the PIMT protein was separately cotransfected into COS-1 cells along with one of the coactivator plasmids (i.e.
Coactivators play a central role in mediating nuclear receptor transactivation by functioning as at least two large multiprotein complexes, one anchored by CBP/p300 and the other by PBP (4, 5). The multistep model of transcription proposes that the acetylation-methylation functions of the initial CBP/p300-mediated complex leads to a transition from CBP/p300-dependent to a mediator-dependent stage of transcription involving the TRAP/DRIP/ARC complex of coactivators anchored by PBP (4, 33). The central importance of CBP/p300 and PBP in transcription is underscored by embryonic lethality observed in gene knockout studies in mice (36–40). One other coactivator of equal importance appears to be the recently cloned PRIP/ASC-2/RAP250/TRBP/NRC (18–22), which has not been identified as part of either the CBP/p300 or the PBP-anchored multiprotein coactivator complexes. However, disruption of the PRIP gene appears to result in embryonic lethality.2 The embryonic lethality of null mutation as well as PRIP’s ability to interact with a variety of nuclear receptors and CBP (18–22) suggested that PRIP is as indispensable as CBP, p300, and PBP in mediating the transcriptional activity of nuclear receptors and other transcription factors (41). To elucidate the functional role of PRIP and to identify coactivator-binding protein(s), we used PRIP as bait in a yeast two-hybrid screen and identified PIMT as a PRIP-interacting protein capable of enhancing PRIP’s coactivator function (41). Interestingly, PIMT binds to S-adenosyl-L-methionine and RNA, implying that PIMT may function as a putative methyltransferase (41). Truncated PIMT, without the methyltransferase domain, still showed its ability to enhance the PRIP-mediated transcriptional activity of PPARγ and RXRα, suggesting that putative enzyme activity of PIMT may not be crucial to transcription under transient transcription conditions (41).

In this study, we have examined the ability of PIMT to interact with other coactivators such as CBP, p300, PBP, SRC-1, and PGC-1α in an attempt to further explore the role of PIMT in nuclear receptor signal transduction. Our results show that PIMT interacts with CBP, p300, and PBP under in vitro and in vivo conditions, but it failed to interact with SRC-1 and PGC-1α, implying selectivity of association. Using GST pull-down assays, we mapped the PIMT binding site(s) on CBP and p300 to their C/H1 and C/H3 domains. Further analysis revealed that the N-terminal of PIMT containing the RNA binding domain interacts with these two sites, whereas the C-terminal S-adenosyl-L-methionine binding domain of PIMT appears to interact with only the C/H3 domain. These two regions, namely C/H1 and C/H3, of CBP/p300 participate in the binding and activity of a variety of transcription factors (26, 45). In particular, a small domain of C/H3 binds diverse proteins including adenoviral E1A oncoprotein and the coactivator TIF-2/SRC-2 (45). The PIMT binding site on PBP was localized to region aa 1101–1200. Since this region is devoid of the LXXLL motif, it appears that binding of PIMT to PBP is not contingent upon the presence of this motif. Nevertheless, the PIMT binding site on PRIP was in the region (aa 773–927) of PRIP that has an LXXLL that is considered necessary for PRIP-nuclear receptor interaction (18, 41).

The in vitro interaction of PIMT with CBP, p300, and PBP was ascertained by immunoprecipitation using antibodies against specific coactivators and then immunoblotting with anti-FLAG to detect the presence of this epitope-tagged PIMT (Fig. 5). Alternatively, the presence of CBP, p300, PBP, and
PRIP were detected by immunoblot analysis of immunoprecipitates obtained using anti-His antibodies to precipitate adeno-virally expressed PIMT with hexahistidine. These approaches clearly established the presence in vivo of PIMT-coactivator complex containing at least four of what appear to be general coactivators. Furthermore, immunofluorescence studies with anti-FLAG revealed the presence of expressed epitope-tagged PIMT protein in a speckled pattern in nuclei. A merging of the distribution patterns of CBP, p300, or PBP, and PIMT in the absence of ligand, was defined as the value 1. The results are the mean of three independent transfection experiments. A, CBP-mediated transfection analysis in CV1 cells. B and C, p300 and PBP-mediated transfection experiments, respectively, in HEK293 cells.

FIG. 7. PIMT represses CBP/p300-mediated transcriptional function but enhances PBP-mediated transactivation activity. Cells were cotransfected with 1.5 μg of PPRE-Luc, 20 ng of pCMV-PPARγ, 0.1 μg of pCMVβ, and 1 μg of additional indicated plasmids in the absence (solid bar) or presence (open bar) of 10−6 M BRL49653. Transfection without the indicated plasmid was compensated by the same amount of pCDNA3.1. Luciferase activity from the transfection without either exogenous CBP, p300, or PBP, and PIMT in the absence of ligand, was defined as the value 1. The results are the mean of three independent transfection experiments. A, CBP-mediated transfection analysis in CV1 cells. B and C, p300 and PBP-mediated transfection experiments, respectively, in HEK293 cells.

FIG. 8. A proposed model in which PIMT functions as a link between the CBP/p300-anchored coactivator complex with the PBP-anchored TRAP/DRIP/ARC complex. CBP/p300 and other proteins in this complex, such as members of the SRC family, possess intrinsic histone acetyltransferase activity, and CARM1 contains histone methyltransferase activity. The TRAP/DRIP/ARC complex anchored by PBP and other proteins such as PRIP with no known enzymatic activities facilitate the recruitment of general transcription factors (GTFs) and RNA polymerase II holoenzyme to initiate transcription of the ligand-bound nuclear receptors.
the multistep model of transcription (4, 5). However, it is plausi-
bility to consider the existence of one major coactivator com-
plex (Fig. 8) and that different signals pass selectively through
different components of this megacomplex to accomplish tran-
scription factor-specific gene transcription instead of the se-
quential recruitment of two complexes. It is important to recall
that SRC-1 with its histone acetyltransferase activity was
found to be dispensable in generating the PPARα-dependent
signal transduction in SRC-1-null mice. This implies a reduc-
dancy of the p160 family of coactivators for PPARα signaling
despite the fact that SRC-1 is an essential component of the
CBP/p300 corepressor complex (46). In contrast, partial blun-
ting of steroid hormone responses was found in SRC-1-null mice
(47), further attesting to the use of different coactivators for
different functions essential for receptor-mediated signal
transduction.

We found that PIMT functions as a strong enhancer of PBP
coaetor function when the PPARγ-mediated transcription
function was assayed using transient transfection in the pres-
ence of PPARγ ligand BRL49653. In contrast, our results also
show that PIMT impairs the CBP/p300 coactivator function.
The integrator protein p300 was demonstrated to interact with
PPARγ and enhance its transcriptional activity (48). The re-
pressor effect of PIMT on the CBP/p300 coactivator function is
opposite of what we observed in this study with PBP and previ-
ously with PRIP (41) where PIMT augmented the coacti-
ator functions of both PRIP and PBP. The mechanism by
which PIMT exerts this coactivator-dependent differential
transcriptional activity is not clear. It is possible that PIMT
by virtue of its ability to bind CBP/p300 may be disrupting the
initial coactivator complex formation in a manner analogous
to that of E1A oncoprotein (44). E1A binding to CBP/p300
inhibes with normal cellular transcription and cell cycle progres-
sion (14). However, the CH3 domain where E1A binds is not
required for the E1A inhibition of transcription. This is due to
the E1A inhibition of the assembly of CBP-nuclear receptor-
coaetator complex formation (44). PIMT also binds to CBP/
p300 at this CH3 site, and overabundance/overexpression of
PIMT or lower concentrations of CBP/p300 may lead to disas-
semble or nonassembly of the CBP/p300-coactivator complex.
Another possibility is that the RNA binding property of PIMT
together with its putative methyltransferase activity, as sug-
gested by its ability to bind S-adenosyl-l-methionine, may in-
fluence transcription or RNA processing machinery. PIMT re-
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