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Inhibition of histone acetyltransferase function of p300 by PKCδ

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Abstract

Protein kinase Cδ (PKCδ) is one of the functionally distinct isoforms in PKC family. p300 is a histone acetyltransferase/transcription coactivator. They share certain properties, such as ubiquitous expression, growth and tumor suppression, and ability to enhance differentiation and apoptosis. In this study, we found that PKCδ but not classical PKC, specifically phosphorylates p300 at serine 89 in vitro and in vivo. This phosphorylation causes inhibition of p300 intrinsic HAT activity. Subsequently, the targeted acetylation of nucleosomal histones is markedly reduced, which causes repression of p300 transcription coactivator function. These findings identify a new signal transduction pathway by which PKCδ may inhibit cell growth and promote cellular differentiation.
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Keywords: CBP; Histone acetyltransferase; Inhibition; p300; Phosphorylation; PKCδ; Transcription

1. Introduction

CBP/p300 is one of the most studied transcriptional coactivators and histone acetyltransferases (HAT). The binding of E1A to CBP/p300 inhibits the HAT and transcriptional activities of CBP/p300 [1–4]. Although the precise mechanism of this inhibition remains unclear, it has been proposed that E1A interacts directly with the CBP/p300 HAT domains as well as disrupts the interactions of associated HATs, such as PCAF [5]. The basic helix–loop–helix protein Twist, implicated in inhibiting differentiation of multiple cell lineages, can directly target the HAT domains of CBP and p300, and inhibit their enzymatic activity [3].

Phosphorylation is known to be an important mode of transcription factor regulation [6]. Phosphorylated p300 has been found in both quiescent and proliferating cells, and the level of p300 phosphorylation changes along the cell cycle progression [7]. Several reports have suggested that CBP/p300 may be phosphorylated by various kinases that are important in either cell cycle regulation or in different signal transduction pathways. Furthermore, these phosphorylation events have been speculated to affect various CBP/p300 activities [8–12]. Recently, the first in vivo phosphorylation site (serine 89) in p300 has been identified [13]. This previous report also showed that PKCα had the strongest activity in HeLa to phosphorylate serine 89 in a p300 fragment, and this serine 89 phosphorylation appeared to repress p300-participated gene expression. However, in above phosphorylation studies, the precise kinase responsible for the specific phosphorylation of p300 in vivo and the physiological significance of the phosphorylation remains unclear.

PKC is a family of serine/threonine kinases that contain the similar regulatory C1 domain structure that responds to the lipid second messenger, diacylglycerol, for activation [14,15]. Different PKC isoforms are unique to each other in their function and so play distinct roles in signal transduction [16,17]. Therefore, it is essential to identify the specific PKC isoform responsible for the phosphorylation in order to understand the physiological significance of the event. We found in the current study that PKCδ, but not PKCα as reported previously, is responsible for the phosphorylation of p300 at serine 89. Further, PKCδ-mediated phosphorylation inhibits p300 intrinsic HAT activity. The same physiological roles of both p300 and PKCδ support our conclusion that they collaborate in the same signal transduction pathway.

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2. Materials and methods

2.1. Protein purification

The flag-tagged p300 proteins (rp300), including wild type and the point mutant at serine 89 to alanine (S89A), were expressed in Sf9 cells using the Baculoviral expression system (BRL Biotechnology) and purified through P11 (Whatman) and anti-flag immuno-affinity (M2-Agarose, Kodak) chromatography. The $6 \times$ His-tagged Gal-CREB fusion protein phosphorylated at serine 133 (P-Gal-CREB) was co expressed in bacteria with PKA catalytic subunit using pET expression system (Novogen). The fusion proteins were then purified by P11 and Ni^{2}-NTA Agarose (Qiagen) chromatography following ammonium sulfate precipitation (25–40%), and analyzed by Western blotting with the anti-CREB phospho-serine 133 antibody.

2.2. In vitro phosphorylation

The purified rp300 protein (100 ng) was phosphorylated by either purified recombinant PKCα (Calbiochem), PKCβII [13], PKCδ (Calbiochem), or PKCθ immunoprecipitated from HeLa cells [18]. The activity of the PKC kinases was verified by phosphorylation using myelin basic protein (MBP, Biomol, 1 μg) as substrate. The phosphorylation reaction was carried out at 30 °C for 20 min in kinase buffer (20 mM Hepes pH 7.4, 10 mM magnesium acetate, 1 mM DTT, and 20 μM ATP) supplemented with 0.5 mM $CaCl_2$, 100 ng/ml PMA, 100 μg/ml phosphatidylserine. After phosphorylation, rp300 and MBP were analyzed by 6% and 12% SDS-PAGE, respectively. For further HAT and transcription assays, phosphorylated rp300 was recovered by using M2-Agarose to separate from the kinases.

2.3. HAT assay

The HAT activity of the purified rp300 proteins (200 ng, or as indicated) was assayed with either free core histones (0.5 μg) or the nucleosomes-assembled plasmid pT3G5 (5 μg) as substrates, and analyzed by the PAGE method [19,20]. The chromatin DNA was incubated with the serine 133-phosphorylated Gal4-CREB fusion protein (P-Gal-CREB) and rp300 before the HAT assays. Transfected flag-tagged p300 and endogenous p300 were prepared by immunoprecipitation from cell lysates with anti-flag or anti-p300 antibody, and assayed for HAT activity with free core histones (0.5 μg) as substrates using the filter-binding method [20].

2.4. Nucleosomes assembly

Nucleosomes were assembled on the plasmid pT3G5 by the octomer transfer method using the short chromatin of HeLa cells as described previously [21–24]. The plasmid pT3G5 was derived from a transcriptional template (pG5HMC2AT) [25] containing a mammalian promoter with upstream Gal4-binding sites, multiple Sp1 sites and a downstream G-less cassette. pT3G5 also contains 10 copies of the nucleosome positioning sequence of 5S rDNA in order to help nucleosome assembly. The nucleosome-assembled DNA was purified by sucrose gradient centrifugation. The quality of the chromatin was analyzed by micrococcal nuclease digestion.

2.5. In vitro transcription assay

Assays were carried out as described previously [21,24,26] except using HeLa nuclear extracts instead of the purified basal transcription factors. Transcription reaction (50 μl) contained 20 mM Tris–HCl (pH 7.9), 100 mM KCl, 0.2 mM EDTA, 3 mM $MgCl_2$, 150 U RNase T1, 0.5 mM ATP, 0.5 mM CTP, 25 μM UTP, 100 μM 3′-*O*-methyl GTP (Pharmacia), 1 μl [a-^{32}P] UTP (NEN, 3000 Ci/mmol, 10 mCi/ml), acetyl CoA (1 μM), TSA (5 μM), HeLa nuclear extracts (50 μg), either P-Gal-CREB (100 ng) or rp300 (400 ng), and the DNA template (pT3G5 plasmid), either naked (100 ng) or reconstituted chromatin (1 μg). A core promoter template (pMLΔ53, 40 ng) was also included in the reaction as a control [27]. After incubation at 30 °C for 60 min, the reaction was stopped by adding 200 μl stop solution (20 mM Tris–Cl, pH 8.0, 1 mM EDTA, 100 mM NaCl, and 1% SDS) and 1 μg tRNA as carrier. After extraction with phenol/chloroform (1:1), RNA was precipitated with ethanol, and analyzed on a 5% polyacrylamide gel containing 8 M urea. After electrophoresis, the gel was soaked in dH_2O, dried, and subjected to autoradiography.

2.6. Transfection and CAT assay

HeLa cells at 50% confluence growing in DMEM/5% fatal calf serum in 10-cm plates were transfected with Lipofectamine (16 μl, BRL) according to the manufacturer's instruction. One or two days after transfection, the cells were lysed in 200-μl buffer (250 mM Tris–HCl, pH 7.4, 1 mM DTT, and 1 mM PMSF) followed by CAT assay using the organic phase extraction procedure [28]. CAT activity was normalized against cotransfected β-galactosidase activity [29].

The transfected cells, after recovery, may be treated either with PMA (100 nM) for 1 h, or with Calphostin C (100 nM) until harvest. HeLa cells expressing either constitutively active (CA, aa 326–672, CAT) or dominantly negative (DN, K368R) mutant of PKCα, or CA (aa 334–674) or DN (K376R) mutant of PKCδ, were prepared by stable transfection as described previously [18]. The stable cell lines were selected in the medium containing 800 μg/ml G418 (BRL), and maintained with 200 μg/ml G418.

3. Results

3.1. PKCδ phosphorylates p300 at serine 89 solely

In previous report [13], two classical PKCs (cPKC), PKCα and PKCβII, and a novel PKC, PKCδ, phosphorylated serine 89 in a small fragment of p300 (rp300n, aa 74–163). In current study, the recombinant full-length p300 proteins (rp300), wild type and the point mutant at serine 89 to alanine (rp300 S89A), were expressed and purified from Sf9 cells (Fig. 1A). In in vitro kinase assays and detection with the phospho-serine 89-specific antiserum, both PKCα and PKCδ phosphorylated serine 89 of rp300 (Fig. 1B). However, PKCδ phosphorylated only wild type rp300 but not the S89A mutant (Fig. 1C), suggesting that PKCδ phosphorylated serine 89 in p300 solely. In contrast, PKCα phosphorylated both wild type and S89A mutant of rp300 (Fig. 1C), indicating that PKCα also phosphorylated p300 residue(s) other than serine 89. As same as PKCα, PKCβII also phosphorylated rp300 at serine 89 and other residue(s) (data not shown).

3.2. PKCδ inhibits p300 intrinsic HAT activity

To examine whether PKC-mediated phosphorylation affects p300 function directly, we carried out HAT assays using free core histones after phosphorylation of the rp300 proteins with either PKCα or PKCδ and further separation of the phosphorylated p300 proteins from the kinase. The HAT activity of wild type rp300 was markedly reduced by the PKCδ-mediated phosphorylation (Fig. 2A, left). PKCδ did not affect the HAT activity of rp300 S89A, indicating that the repression of p300 HAT activity is specific to the phosphorylation at serine 89. Interestingly, after phosphorylation with PKCα, the HAT activity of the S89A mutant was increased (Fig. 2A, right), suggesting that PKCα-mediated phosphorylation at the other putative residue(s) favors p300 HAT function. On the other hand, wild type p300, after phosphorylation at both serine 89 and the other putative residues by PKCα, was not affected in its HAT activity. This result

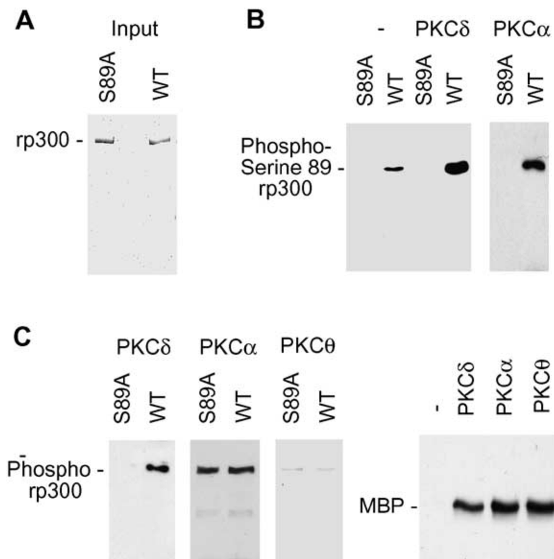

Fig. 1. PKCδ phosphorylates full-length p300 at serine 89 solely. (A) Coomassie staining of recombinant p300 (rp300) proteins, wild type (WT) and the serine 89 point mutant (S89A). The rp300 proteins were expressed in and purified from Sf9 cells. (B) Western blot analysis of the rp300 proteins with anti-phospho-serine 89 antiserum after phosphorylation by either PKCδ or PKCα. (C) Autoradiograph demonstrating phosphorylation of rp300 (left) and MBP (1 μg; right) by different isoforms of PKC.

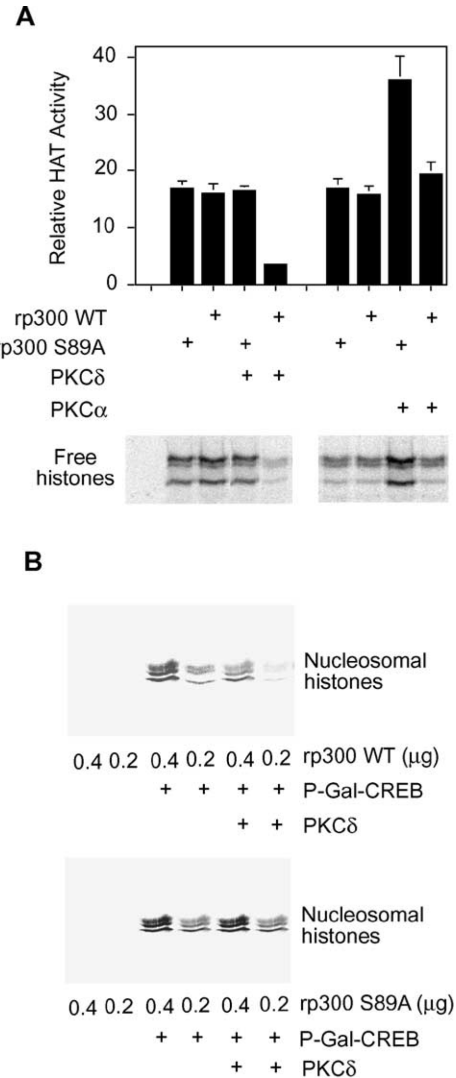

Fig. 2. Inhibition of p300 HAT activity via phosphorylation at serine 89 by PKCδ. (A) The rp300 proteins were expressed in and purified from Sf9 cells. The rp300 preparation has been optimized to have a similar background in HAT activity between the wild type and the S89A mutant. The rp300 proteins treated with kinases were purified further to separate from the kinases. Fluorographs are shown at the bottom, indicating acetylation of free core histones by rp300 with and without phosphorylation by either PKCα or PKCδ. The HAT assay is analyzed quantitatively with a phosphoimager (top graph). Error bars represent the standard deviation ($n = 3$). (B) HAT assays for rp300, either wild type (WT) or the serine 89 mutant (S89A), with or without phosphorylation by PKCδ in the presence or absence of Gal-CREB fusion protein phosphorylated at serine 133 (P-Gal-CREB). The pT3G5 chromatin was used as substrate in the assays.

indicates that the inhibitory effect of the serine 89 phosphorylation and the stimulating effect of the non-serine 89 phosphorylation were neutralized by each other.

When we used nucleosomes-assembled plasmid chromatin (pT3G5) as substrates in HAT assays, the results were identical to those using free histones (Fig. 2B). In addition, efficiency of rp300 to acetylate nucleosomes depended on activator Gal-CREB phosphorylated at serine 133 (P-Gal-CREB) (Fig. 2B), which bound to the nucleosomal DNA through the Gal4 sites and recruited rp300 onto the chromatin [4]. This p300 targeting for acetylation is similar to the previous observation that GCN5 HAT protein and transcription coactivator targeted acetylation of nucleosomal histone [24]. Importantly, serine 89 phosphorylation by PKCδ reduced the ability of p300 to target acetylation of nucleosomes.

3.3. Overall PKC does not inhibit p300 HAT activity in vivo

We further carried out functional assays in vivo. The expression vectors for the flag-tagged p300, both wild type and the S89A mutant, were transfected into HeLa cells, respectively. After transfection, cells were either untreated or treated with PKC activator phorbol 12-myristate 13-acetate (PMA) or with PKC-specific inhibitor Calphostin C. The flag-p300 proteins were recovered by immunoprecipitation with anti-flag antibodies. Both wild type and S89A rp300 proteins expressed in similar amounts in all treated and untreated cells (Fig. 3A, bottom), suggesting that PMA and Calphostin C had no effect on expression of rp300. PKC activator PMA increased while the inhibitor Calphostin C decreased the level of p300 phosphorylation at serine 89 (Fig. 3A, middle), confirming that PKC mediates the serine 89 phosphorylation in vivo. The reduction of the serine 89 phosphorylation of p300 was also observed in the cells treated with either Go6976 (50 nM) or Rottlerin (30μM) (data not shown), implying that both cPKCs and PKCδ mediate in vivo phosphorylation of serine 89.

In normal HeLa cells, the p300 S89A point mutant was more active than the wild type p300 in acetylation of histones (Fig. 3A, top left), suggesting that phosphorylation at serine 89 inhibited p300 HAT function in vivo, just as it did in vitro. The HAT activity of the p300 mutant S89A was increased significantly in PMA-treated cells (Fig. 3A, top middle). In this case, the mutant did not have any serine 89 phosphorylation but should have an increased phosphorylation level at the putative non-serine 89 residues because PMA activates PKCα, too. Similar to the in vitro situation (Fig. 2A), non-

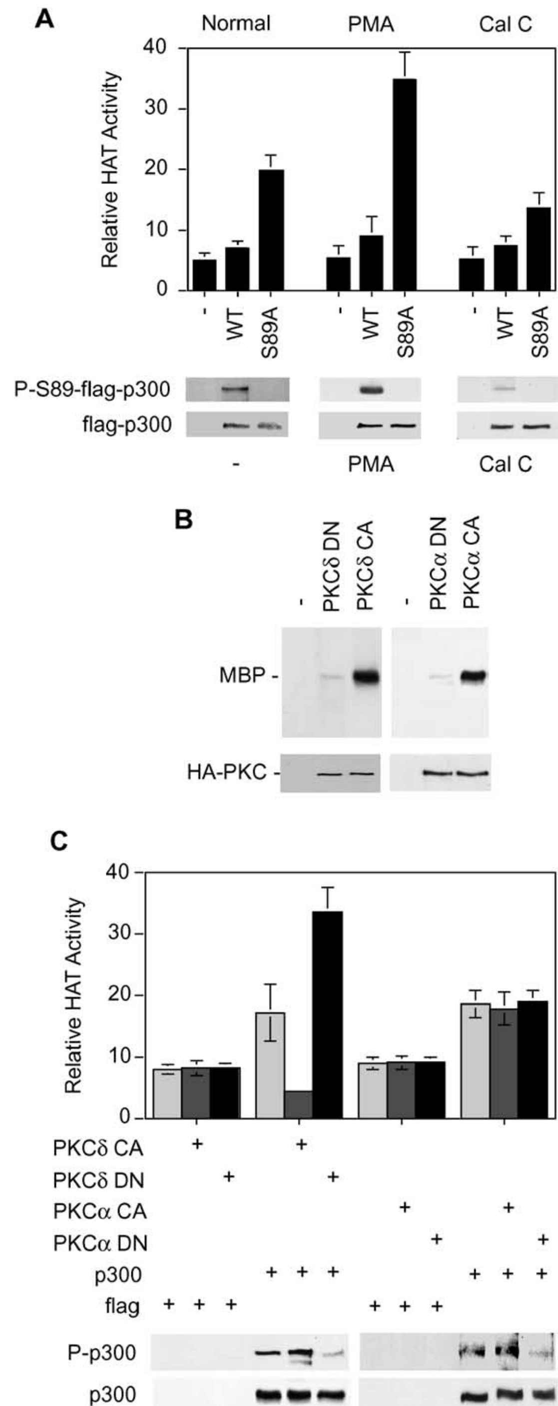

Fig. 3. PKCδ mediates phosphorylation of p300 at serine 89 and subsequent inhibition of HAT activity in vivo. (A) HeLa cells were transfected transiently with the flag-tagged p300 constructs, either wild type (WT), or the serine 89 point mutant (S89A), or the empty vector (–). After transfection, cells were either untreated (Normal) or treated with PMA or Calphostin C (Cal C). The flag-p300 proteins were recovered by immunoprecipitation with antibody against the flag tag, analyzed by HAT assays (top), and by Western blotting (bottom) with antiserum against phospho-serine 89 (P-S89-flag-p300) and antibody to p300 (flag-p300). (B,C) HeLa cells were transfected stably with PKCα or PKCδ constitutively active (CA) or dominantly negative (DN) mutant. The mutant proteins were immunoprecipitated with antibody against the HA tag, analyzed by Western blotting with antibody against either PKCα or PKCδ (bottom), and assayed for kinase activity with MBP as substrate (top) (B). The endogenous p300 proteins were immunoprecipitated with the antibody to p300 from different cells, and analyzed by HAT assays (top) and by Western blotting (bottom) with the antiserum against phospho-serine 89 (P-p300) and the antibody to p300 (p300) (C). The immunoprecipitation with the antibody to the flag tag (flag) was used as a control. HAT activity was assayed using free core histones as substrates and normalized against total proteins. Error bars represent the standard deviation ($n = 3-5$).

serine 89 phosphorylation had stimulating effect on the p300 HAT activity. In the cells with different treatment, phosphorylation level at serine 89 of the wild type rp300 was increased by PMA and reduced by Calphostin C while the HAT activity was not affected significantly by the changes of the serine 89-phosphorylation level (Fig. 3A). This may be resulted at least in part by the simultaneous change of PKCα-mediated phosphorylation level at the other putative residues, which neutralizes the inhibitory effect of the serine 89 phosphorylation as we observed in vitro.

3.4. PKCδ mediates repression of p300 HAT in vivo

To investigate whether PKCδ mediates repression of the p300 HAT activity in vivo, we used HeLa cells stably expressing either CA or DN mutant of PKCα or PKCδ [18]. The specificity of the enzymatic reaction for these CA and DN mutants in the transfected cells is shown in Fig. 3B, which is consistent with the previous observation [18]. The levels of p300 in all PKCα and PKCδ CA- and DN-expressing cells were similar, and no significant difference from that in normal HeLa cells (Fig. 3C, bottom), suggesting that these PKCα and PKCδ mutants do not affect the expression of p300. As expected, both PKCα and PKCδ CA mutants increased and both DN mutants reduced the phosphorylation level at serine 89 (Fig. 3C, bottom). In the cell cotransfected with PKCδ CA mutant that over-expressed PKCδ activity, the p300 HAT activity was inhibited while the HAT activity was enhanced in the cell overexpressing PKCδ DN mutant (Fig. 3C, top). In contrast, in the cell cotransfected with either PKCα CA or DN mutant, the p300 HAT activity was not affected (Fig. 3C, top). These results indicate that PKCδ, rather than PKCα, is responsible directly for the inhibition of the p300 HAT activity in vivo. The difference in the p300 HAT activity between the cells expressing either mutant type of PKCα and PKCδ, respectively, did not reflect their similar phosphorylation levels at serine 89 (Fig. 3C). Similar to the situations in vitro and in the PMA or Calphostin C-treated cells, this result can be explained by the facts that PKCδ phosphorylates p300 only at serine 89, resulting in inhibition of the p300 HAT activity while PKCα phosphorylates both serine 89 and non-serine 89 residues, which neutralize the effect on p300 HAT. The expression of PKCα CA mutant increased while the expression of PKCα DN mutant decreased phosphorylation of both serine 89 and the other putative residues, therefore each resulting neutral effect on the p300 HAT activity. Because the rp300 proteins used in the HAT assays were prepared from cells by immunoprecipitation, the measured HAT activity may be affected by change in the association of p300 with other HAT proteins. The measured HAT activity of p300 might also be affected by the PKC mutants in serine 89-phosphorylation-independent ways. In summary, our data suggest that PKCδ plays a major role in the serine 89-phosphorylation-dependent repression of p300 HAT activity in vivo.

3.5. PKCδ inhibits transcription on chromatin

Further in vitro transcription assays using the transcription template pT3G5, either naked DNA or reconstituted chromatin, were carried out in HeLa nuclear extracts supplemented with either P-Gal-CREB or rp300 proteins. Tran-

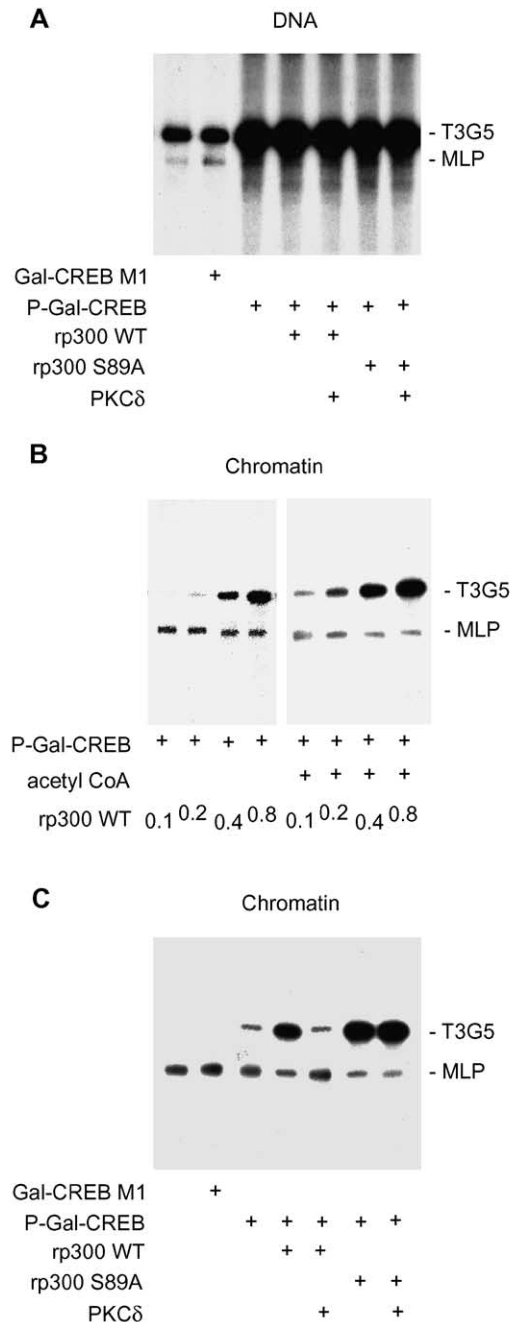

Fig. 4. PKCδ inhibits p300 transcription coactivator function in vitro. In vitro transcription assays were carried out on the naked (A) and chromatin (B,C) templates (pT3G5). The reactions contain, as indicated, the Gal4-CREB fusion protein phosphorylated at serine 133 by PKA (P-Gal-CREB), Gal4-CREB bearing a point mutation at serine 133 (Gal-CREB M1), and rp300, either wild type (WT) or serine 89 point mutant (S89A) with or without phosphorylation by PKCδ. A major late core promoter (MLP) template (pMLΔ53) was included as a control in all reactions.

scription was activated vigorously on the naked template in the presence of P-Gal-CREB and regardless of the addition of the rp300 protein and the PKCδ treatment (Fig. 4A). In contrast, transcription on the chromatin template was repressed severely in the absence of the additional rp300, and the transcription could be derepressed markedly by the addition of either wild type rp300 or S89A mutant in the reaction (Fig. 4C). An initial titration for p300 in transcription on chromatin indicated that acetyl CoA enhanced p300-participated and chromatin-dependent transcription (Fig. 4B), which was consistent with previous observation [21,30]. When the additional wild type rp300, but not when the S89A mutant, was phosphorylated by PKCδ, the transcription derepression was reduced markedly (Fig. 4C). These observations indicated that the phosphorylation at serine 89 caused the deregulation of transcription. The inhibition of the transcription derepression on chromatin by the phosphorylation is consistent with the repression of the acetylation targeted by p300 after the same phosphorylation event (Fig. 2B). Our observations suggest that the deregulation of p300-participated transcription is associated with the inhibition of the HAT activity.

3.6. PKCδ mediates coactivator repression in cells

To examine whether this PKCδ-mediated transcription regulation event also occurs in vivo, a reporter system driven by the CRE enhancer (p(-71)SRIF-CAT) was transfected into HeLa cells along with the expression vectors for CREB and PKA catalytic subunit. The empty control vector or the expression vector for wild type p300 or S89A point mutant was cotransfected. HeLa cells used in these experiments were either wild type or the cells stably expressing PKCδ CA or DN mutant. The PKA-induced transactivation from the CRE enhancer was repressed by PKCδ CA mutant but enhanced by PKCδ DN mutant (Fig. 5). PKCδ CA mutant enhanced the phosphorylation of p300 at serine 89 and resulted in inhibition of its HAT activity, while PKCδ DN mutant inhibited the phosphorylation (Fig. 3C). These observations suggest that the effect on the transactivation of the reporter gene by the PKCδ mutants was associated with the phosphorylation-dependent alternations of p300 HAT activity. When the p300 point mutant was cotransfected, the reporter CAT activity was not affected significantly by the expression of either PKCδ mutant (Fig. 5), indicating that this PKCδ-mediated transcription inhibition event in vivo was also specific to the phosphorylation of p300 at serine 89.

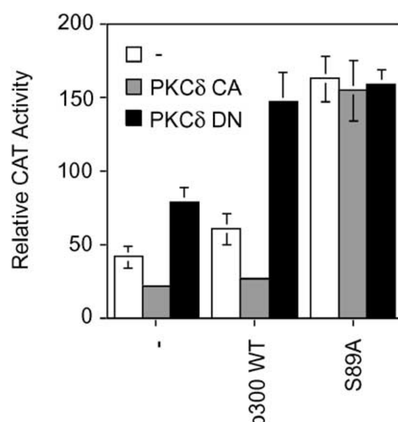

Fig. 5. PKCδ inhibits p300 transcription coactivator function in vivo. HeLa cells with stable expression of either none (−), PKCδ CA, or PKCδ DN mutant were transfected with the CRE reporter p(-71)SRIF-CAT (1 μg), pRc/RSV-CREB (0.5 μg), and pRc/RSV-PKA (0.25 μg). The expression vector for p300 (0.5 μg), either wild type (WT) or the serine 89 point mutant (S89A), was cotransfected as indicated. CAT activity was normalized against cotransfected β-galactosidase activity. Error bars represent standard deviation ($n = 3-5$).

4. Discussion

In summary of our current study, PKCδ is responsible for the phosphorylation of p300 at serine 89 and subsequent repression of the transcription coactivator function. Mechanically, this PKCδ-mediated repression of p300 function results from direct inhibition of p300 intrinsic HAT activity by the serine 89 phosphorylation. This study represents the first detailed analysis of the mechanism involved in the regulation of a transcription pathway through phosphorylation of a coactivator.

Previous reports have suggested that the amino terminus of CBP/p300 is not required for p300 HAT activity and is dispensable, at least with some activators, for p300 coactivator activity [31,32]. In these two studies, they used the N-terminal deletion mutants of CBP/p300. In contrast, in our studies using the p300 wild type and point mutant from serine 89 to alanine (S89A), we observed reduced levels of the p300 HAT and coactivator activities upon the serine 89 phosphorylation. Phosphorylation at serine 89 may induce a conformational change of p300 that hinders its HAT and transcriptional function. The S89A point mutant prevents p300 from being phosphorylated and the subsequent inhibitory conformational change, and may still conserve the normal p300 function. As a result, under the normal phosphorylation condition, the S89A point mutant is more active than wild type p300 in acetylation and transcriptional coactivation. On the other hand, the N-terminal deletion mutants may cause a different conformational change of p300, but which may not alter the HAT activity and the coactivator function. Detailed structural studies will be able to better resolve this question in the future.

The phosphorylation of p300 at serine 89 has been detected in several different types of cells [13] while PKCδ is expressed ubiquitously [14,33], suggesting that this phosphorylation-dependent regulation of the transcription pathway may not be tissue-specific. Because this phosphorylation inhibits p300 intrinsic HAT activity directly, it may also affect p300 transcription coactivator function in different systems [13]. These observations suggest that the PKCδ-mediated serine 89 phosphorylation may have a broader

effect on the p300-participated transcription events. The regulation of different transcription pathways by the PKCδ may not only go through the inhibition of p300 targeting of nucleosomal histone acetylation, but also, possibly, through inhibiting p300 acetylation of other transcriptional factors [34]. In addition, we should emphasize that the present studies do not exclude the possible roles of other isoforms of PKC in modulating the function of p300 in various cell types and at certain stages during the cell cycle.

Several important biological functions specific to PKCδ isoform have been reported, such as regulation of G1 cyclin expression [35], potential growth/tumor suppressor [36], and promotion of differentiation and apoptosis [37,38]. CBP/p300 shares these important biological functions with PKCδ. These facts support our finding that PKCδ and CBP/p300 function collaboratively in the same signal transduction pathway, which may result in the suppression of growth and the promotion of differentiation. PKCα is a dominant PKC isoform in HeLa cells, and was thought previously to contribute together with PKCδ to serine 89 phosphorylation and subsequent inhibition of p300 coactivator activity [13]. In fact, we now show that effect of PKCα-mediated phosphorylation on the p300 HAT activity is neutralized by phosphorylations at serine 89 and the other putative residues. In vivo PKCα could compete with PKCδ to phosphorylate p300 and interfere with the inhibitory effect of PKCδ on the p300 HAT activity. Thus, the inhibitory effect on p300 function by serine 89 phosphorylation should be balanced by relative levels of activated PKCα and PKCδ. It is known that PKCα and PKCδ function differently during the cell cycle [39]. Difference in dynamics of their activities at G1 phase can also be observed (Yuan, unpublished data). Because p300 plays important regulatory roles at G1/G0 transition and during further differentiation [1], this difference in the timing of their activation may provide the basis for the physiological role of serine 89 phosphorylation of p300. Further study of this phosphorylation event during the cell cycle and during the process of differentiation should enrich our understanding of our proposed PKCδ/p300-mediated signal transduction pathway.

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