The Human Proliferating Cell Nuclear Antigen, PCNA, Regulates Transcriptional Coactivator p300 Activity and Promotes Transcriptional Repression

Running Title: PCNA regulates p300 activity and transcription

Rui Hong and Debabrata Chakravarti*

Department of Pharmacology
University of Pennsylvania School of Medicine
Philadelphia, PA 19104-6084

*Corresponding author:
810 BRBII/III
421 Curie Blvd
Department of Pharmacology
University of Pennsylvania School of Medicine
Philadelphia, PA 19104-6160
Tel: 215-573-8470
Fax: 215-573-9004
Email: debu@pharm.med.upenn.edu
Summary:

Chromatin structure plays an important role in DNA replication, repair and transcription. p300 is a transcriptional coactivator with protein acetyltransferase activity, and PCNA plays important roles in DNA replication and repair. It has been recently shown that p300 is necessary for DNA synthesis and repair. However it is not known whether human PCNA, in a reciprocal manner, can regulate the enzymatic activity and transcriptional regulatory properties of p300. Here we show that human PCNA associates with p300 and potently inhibits the acetyltransferase activity and transcriptional activation properties of p300. Surprisingly, PCNA fails to inhibit PCAF acetyltransferase function as well as PCAF dependent transcription. Additionally, PCNA potently represses transcription when targeted to chromatin in vivo. Consistent with these observations, using chromatin immunoprecipitation assays, we demonstrate that PCNA recruitment to promoters causes hypoacetylation of chromatin. Together, our results demonstrate for the first time a novel role for human PCNA in transcriptional repression and in modulating chromatin modification. The reciprocal modulation of p300 and PCNA activities by each other provides an example of integrative regulatory crosstalk among chromatin-based processes such as DNA transcription, repair and synthesis.
Introduction

Chromatin structure plays an important role in the regulation of transcription. The basic subunit of chromatin, the nucleosome core, is a complex of DNA and histones. Acetylation of the lysine residues of histone tails is generally involved in transcriptional activation processes (1,2). In agreement with this observation, many transcription coactivators were shown to possess intrinsic histone acetyltransferase (HAT) activity, providing a mechanism for transcriptional activation by these groups of coactivators. p300/CBP and PCAF represent two groups of extensively studied coactivators/HATs. p300 was first discovered as a protein that interacts with the adeno-viral transforming protein E1A while CBP was originally identified as a CREB (cAMP response element-binding protein) binding protein (3-5). Subsequently, p300/CBP have been demonstrated to interact with a wide variety of proteins and to function as transcriptional scaffolds. They can facilitate transcriptional initiation by recruiting other coactivators such as p160 group of proteins, PCAF and RNA polymerase holoenzyme (6-9). p300/CBP can also modify chromatin through their intrinsic HAT activity (10,11). The importance of p300/CBP is well illustrated by their roles in cell proliferation and differentiation (3,12,13). PCAF (p300/CBP-associated factor) is another coactivator with intrinsic HAT activity. It shares homology with GCN5-HAT in yeast and is required for nuclear receptor-mediated transcription, cell differentiation as well as muscle differentiation (14,15). In addition, studies have demonstrated that the HAT activity of p300/CBP and PCAF is necessary for their coactivator function in modifying chromatin structure during transcription and other cellular processes (13,16).

PCNA (proliferating cell nuclear antigen) is a homologue of the β subunit of the
prokaryotic DNA polymerase and well known as a processivity factor for the eukaryotic DNA polymerses d and e (17). PCNA clamps onto DNA and slides along it during replication (18). As a highly conserved replication factor, PCNA interacts with a large number of proteins, most of which are involved in DNA biosynthesis processes such as replication and repair. For example, PCNA interacts with the flap endonuclease-1 (Fen1) and DNA ligase I, both of which function in processing the primer RNA and joining Okazaki fragments during DNA replication (19,20). PCNA also interacts with the components of DNA mismatch repair MLH1 and MSH2 (21) as well as those of nucleotide excision repair including DNA polymerase e, replication factor C and replication protein A (22,23). It also interacts with the DNA methyltransferase MCMT, marking newly replicated DNA (24). More recently, PCNA has been shown to bind to chromatin assembly factor CAF-1, facilitating the assembly of chromatin at the replication fork (25). Other PCNA-interacting proteins play important roles in the regulation of cell cycle. Among them are cdk/cyclins, a cdk/cyclin inhibitor p21 (26) and several proteins induced by cell cycle arrest or DNA damage such as Gadd45 and MyD118 (27,28). PCNA itself is a cyclin (29) and is highly up regulated in S-phase. These observations have been utilized in clinical diagnosis where PCNA serves as a biomarker for tissue malignancy or regeneration (30,31). Therefore, PCNA can function as a “bridging molecule” that targets proteins with distinct roles into DNA-based processes.

Besides being a necessary factor for processes of DNA metabolism, PCNA has recently been implicated in epigenetic inheritance and transcription. For example, by recruiting CAF-1, PCNA marks the nascent DNA, providing a mechanism for imprinting (25,32). In *S. cerevisiae*, PCNA is involved in gene silencing through CAF-1 dependent and independent mechanisms (32). In *Drosophila*, mutation of PCNA suppresses position effect variegation (PEV) (33),
indicating its role in transcriptional repression and heterochromatin function. In mammals, PCNA was shown to interact with histone deacetylase 1 (HDAC1), implying a role for histone hypo-acetylation in DNA replication (34). These results demonstrate that the function of PCNA expands beyond DNA replication and repair and may have a critical role in epigenetic events and transcription. However, little is known about transcriptional regulatory roles of human PCNA.

Recently, Hasan et al. demonstrated that the transcriptional coactivator p300 might be involved in DNA repair-synthesis through its interaction with PCNA, illustrating the coordination between these two versatile proteins (35). Consistent with this observation, p300 was shown to interact with PCNA and the endogenous p300-PCNA complex promoted DNA synthesis in vitro. Moreover, p300 associated with newly synthesized DNA after UV irradiation. These results led the authors to suggest that p300’s chromatin remodeling ability might facilitate PCNA function at DNA lesion sites although a direct role for the HAT activity of p300 in DNA repair-synthesis was not demonstrated. Another piece of evidence supporting the role of p300 in DNA metabolism is the discovery that p300 can form a complex with and acetylate Fen1. Acetylation of Fen1 by p300 impaired the DNA binding and nuclease activity of Fen1 (36). Conversely, the above results raised an interesting possibility that PCNA might regulate the chromatin remodeling and transcriptional regulatory properties of p300/CBP and/or other nuclear HATs/coactivators.

Activities known to regulate HATs can be divided into two groups based on their targets. In one group, the acetylase enzymes are targeted by cellular or viral interacting proteins (e.g., inhibition of p300/CBP HAT activity by E1A and PU.1’s (37,38)), or by post-translational modification (e.g., the stimulation of CBP-HAT activity by phosphorylation (39)). On the other
hand, the regulatory activity can target the substrate histones (40-42). This is illustrated by the INHAT complex, which we recently purified and characterized. This complex binds to histones and plays a role in chromatin modification by blocking the access of HATs to their histone substrates, a mechanism referred to as histone masking (41). The subunits of INHAT identified as TAFIα, TAFIβ/Set and pp32, bind to histones in vitro and in vivo. Based on our results we proposed INHAT to be a component of the repressive state of chromatin (41,43). During the initial purification of INHAT, we noticed that several proteins consistently co-purified with INHAT. One of these proteins was identified as PCNA. The co-purification of PCNA with INHAT as well as the involvement of the p300-PCNA complex in DNA repair prompted us to explore the potential role of human PCNA in the regulation of histone acetylation by p300/CBP and PCAF and subsequently, its role in transcription.

In this paper, we provide data from in vitro experiments and cell based transfection studies, in which we examined the effect of PCNA on p300 or PCAF activity. Our results demonstrate that PCNA is a potent inhibitor of the HAT activity of p300 in vitro and of p300/CBP dependent transcription including nuclear hormone receptor, RAR function in vivo. Surprisingly, PCNA does not inhibit PCAF-HAT activity and fails to block PCAF dependent transcription. Furthermore, in contrast to INHAT, which regulates histone acetylation by binding to histones, PCNA functions by associating with the enzyme p300 but not the HAT substrate histones in vitro. Consistent with these observations, we show that endogenous PCNA interacts with p300 but not PCAF in cells. Finally, when targeted to a promoter, PCNA can reduce the level of histone acetylation and repress transcription in vivo. While the physiological implications of this differential HAT-inhibition by PCNA remain to be elucidated, our results implicate a novel regulatory role for human PCNA in chromatin modifications and in
transcriptional regulation.

Experimental Procedures

Plasmids and Proteins

For bacterial and eukaryotic expression constructs of PCNA and its mutants, the appropriate PCR-amplified fragments were cloned into pGEX4T2, pCMX-PL1, pHRN-CMX-PL1, pCMX-GAL4 vectors. Sequences of all constructs surrounding the cloning sites were verified by automated sequencing. Recombinant GST fusion proteins were purified using glutathione beads (Amersham Biosciences). Baculovirus-expressed Flag-tagged p300 and Flag-tagged PCAF was obtained as described (44). INHAT complex was purified from HeLa nuclear extracts as described (41).

HAT Assay

Histones were either purified (a kind gift of S. Kultsev) or obtained commercially (Roche Molecular Biochemicals). HAT assays were performed in the presence of 10 mM sodium butyrate as described (41). Reaction products were separated by SDS-PAGE and analyzed by phosphorimager.

In Vitro Immunoprecipitation and Interaction Assays

\[^{35}\text{S}]\] methionine-labeled PCNA and pp32 were synthesized using their pCMX constructs in a coupled transcription-translation system (TNT, Promega), and incubated with approximately equal amounts of beads-bound p300, PCAF or histones (immobilized onto beads by pan-histone antibodies) for 30 min at room temperature in buffer containing 150 mM KCl, 20 mM Tris-HCl
(pH 7.6), 5 mM MgCl₂, 1 mM DTT, and 10 µg/ml BSA. After extensive wash, bound proteins were separated by SDS-PAGE and analyzed by phosphorimager.

**Transfection Assay**

NIH3T3 or 293T cells were seeded in a 48-well dish and transfected by liposome-mediated transfer (Lipofactamine 2000, Invitrogen) with internal control plasmid pRLSV40 (5 ng), gal4-TK-luc reporter plasmid (100 ng), pCMX-GAL4-RARα (20 ng), or pCMX-GAL4-p300 plasmid (100 ng) in the absence or presence of the pCMX-PL1 constructs of PCNA where indicated (43). The amount of DNA in each transfection was kept constant by addition of intact pcDNA3 vector. The media was replaced approximately 6 hr following transfection with fresh Dulbecco’s Modified Eagles Medium with 1 µM all trans retinoic acid (ATRA) where indicated. 48 hours after transfection, cells were harvested and lysed for dual luciferase assays (Promega). Transfection of 293T cells with chromosomally integrated gal4-TK-luc or gal4-SV40-luc reporters was carried out essentially as above except that only GAL4 constructs were transfected into cells and cells were only assayed for firefly luciferase activity. For all figures, each value is the mean of six replicates from a single assay. The results shown are representative of at least three independent experiments. Error bars indicate standard deviations.

**Coimmunoprecipitation**

Briefly, HeLa cell nuclear extract was pre-cleared by incubating with protein A or G Sepharose beads at 4 °C for one hour. Pre-cleared extract was subsequently incubated with primary antibody at 4°C overnight with gentle agitation. Then protein A or G Sepharose beads were added and incubated for one hour. The immunoprecipitates were collected by centrifugation and washed extensively with an IP buffer (50 mM Tris-HCl (pH8.0), 150 mM
NaCl, 10% glycerol, 1mM PMSF and 0.5% Triton X100). Bound proteins were re-suspended in SDS-PAGE sample buffer and subject to SDS-PAGE and immunoblotting. Antibodies used include: anti-p300 (Upstate Biotechnology 05-267 or Santa Crutz sc-585), anti-PCAF, anti-PCNA (Santa Crutz sc-56) or control antibody mouse IgG (Vector). Immuno-blotting was performed with anti-TAFII15 (kindly provided by Dr. R.G. Roeder of Rockefeller University, New York), anti-PCNA, anti-p300, or anti-PCAF antibodies.

**Immunohistochemistry**

Cells were fixed with methanol/acetone (1:1 v/v) at room temperature for 2 minutes and rinsed briefly with PBS. After a 30-minute incubation in PBS/3%BSA (blocking solution), cells were incubated with primary antibodies in blocking solution for 2 hours. Cells were then washed with blocking solution three times with 5 minutes each and incubated with the secondary antibody for 1 hour. After washing, cells were stained with 300 nM DAPI (Sigma) in PBS before mounting. Images were captured utilizing a BioRad confocal microscope. Primary antibodies include: anti-PCNA (Santa Crutz sc-56), anti-p300 (Santa Crutz sc-585) and anti-PCAF (kindly provided by Dr. Y. Nakatani of Dana-Farber Cancer Institute, Boston or from Upstate Biotechnology 07-141). Secondary antibodies include cyanine Cy3-conjugated donkey-anti-mouse antibody (Jackson ImmunoResearch Laboratories 715-165-150) and fluorescein FITC-conjugated goat-anti-rabbit antibody (Jackson ImmunoResearch Laboratories 111-095-144).

**Chromatin Immunoprecipitation (ChIP)**

ChIP was carried out as essentially described in protocols from Upstate Biotechnology (45,46). Briefly, 293T cells were transfected with 10ug of DNA and harvested 48 hours later.
Cells were crosslinked with 1% formaldehyde in the medium at 37°C for 10 minutes, and then scraped into SDS lysis buffer. Samples were further sonicated and diluted for immunoprecipitation with antibodies as indicated. The immunoprecipitates were eluted and crosslink reversed. DNA fragments were purified using Qiagen PCR purification kit and then amplified by PCR reaction for quantitation. Anti-acetyl-histone H3 and anti-acetyl-histone H4 antibodies are purchased from Upstate Biotechnology. Primers utilized for gal4-TK promoter analysis were: 5’ -aggttaacttatgttcccc- 3’ and 5’ -ctttatgttttggttc- 3’. Primers used for gal4-SV40 promoter analysis were: 5’ -gtacttatgtttaac- 3’ and 5’ -ctttatgttttggttc- 3’(47). Each experiment was repeated at least twice with similar results acquired. Results shown are the representative of the data.
Results

PCNA selectively inhibits the HAT activity of p300 in vitro

Since PCNA partially co-purified with INHAT, we decided to determine its role in p300-mediated histone acetylation. For that purpose, core histones were incubated with $^{14}$C-labeled acetyl-CoA and increasing amounts of purified GST-PCNA in a p300-mediated HAT assay system. As a control and observed previously, INHAT blocked p300-mediated histone acetylation (Figure 1A, lanes 3 and 4). A progressive inhibition of p300-mediated core histone acetylation was observed with increasing amounts of GST-PCNA (Figure 1A, lanes 5-8). Purified GST alone even at the highest concentration tested had no effect on the assay (lane 2). No proteolytic degradation of histones in the presence of GST-PCNA or INHAT was observed (data not shown). In addition, the involvement of any deacetylases was excluded by the use of HDAC inhibitor, sodium butyrate, as well as the bacterially and baculovirus-expressed and purified recombinant proteins in these in vitro assays. The lysine residue specificity of this inhibition was examined with the available site-specific anti-acetyl-histone antibodies. PCNA inhibited acetylation of histone H4 lysine 8, histone H4 lysine 12 and histone H3 lysine 14 at varying degrees, with the strongest inhibition on the acetylation of histone H4 lysine 8 (Figure 1B). Interestingly, the acetylation of H4 lysine 8 is well known to be involved in transcriptional activation (48).

Our previous studies demonstrated that the INHAT complex could block histone acetylation by p300/CBP as well as by PCAF. To explore whether PCNA regulates PCAF-mediated histone acetylation and also to determine the target specificity of PCNA, HAT assays were performed utilizing purified histone H3 and PCAF. PCAF prefers to acetylate histone H3
in vitro (Figure 1C, lane 1) (49,50). Surprisingly, GST-PCNA failed to inhibit histone H3 acetylation by PCAF (Figure 1C) under the same conditions where the same amounts of GST-PCNA led to a dramatic inhibition of histone acetylation by p300 (compare Figure 1A, lane 8 with Figure 1C, lane 8). If any, PCNA slightly stimulated PCAF mediated histone acetylation. As a control and in agreement with published results, the INHAT complex inhibited PCAF-mediated histone H3 acetylation (lanes 3 and 4) (41). From these experiments, we conclude that PCNA can selectively inhibit histone acetylation by p300, but not by PCAF in vitro.

**PCNA binds to p300 but not to PCAF in vitro and in vivo**

The selective inhibition of p300 activity by PCNA prompted us to explore whether a potential differential interaction between PCNA and p300 or PCAF exists. For that purpose, we first carried out in vitro binding assays utilizing PCNA and core histones, p300, or PCAF (Figure 2A). \[^{35}\text{S}]\)-labelled PCNA was incubated with approximately equal amounts of beads-bound recombinant Flag-tagged p300 or PCAF. PCNA was also incubated with histones and immunoprecipitated with pan-histone antibodies to determine whether PCNA may block p300 HAT activity by binding to histones. Bound proteins were then separated by SDS-PAGE and analyzed by phosphorimager. Little interaction was observed between PCNA and histones. As a control, the INHAT subunit pp32, bound strongly to histones but not to p300 or PCAF as previously observed (compare lane 7 with lane 8) (41). Importantly, while PCNA bound p300 (lane 3), little or no interaction was observed with PCAF (lane 5). Therefore, PCNA selectively interacts with p300 but not PCAF or histones in vitro.

The region of p300 that mediates the interaction between PCNA and p300 has been mapped to the C-terminal region of p300 outside its HAT domain (35). But the corresponding
region on PCNA has not been determined. For this purpose, three truncation mutants that evenly span the length of human PCNA, P1 (a.a. 1-88), P2 (a.a. 89-178) and P3 (a.a. 179-261) were generated (Figure 2B, right panel). Because of the small size of each fragment, for the interaction assay, we used $[^{35}\text{S}]$-labelled GAL4 DNA binding domain (GAL4-DBD) fusion of the mutants (Figure 2B, lower panel, marked with ‘*’) and incubated each with beads-bound Flag-p300. As controls, both GAL4-full-length-PCNA and GAL4-DBD alone were also included. After wash, bead-bound proteins were subjected to SDS-PAGE and retention of labelled proteins analyzed by phosphorimager. As shown in Figure 2B, GAL4 protein itself did not bind to p300 (lane 1) while GAL4-full-length-PCNA bound to p300 (lane 2) as observed in (A). GAL4-PCNA-P1 (lane 3) as well as GAL4-PCNA-P3 (lane 5) bound to p300 while GAL4-PCNA-P2 (lane 4) failed to bind to p300 in these in vitro binding assays. Thus our mapping study indicated that at least two regions of PCNA interact with p300 in vitro. Interestingly, crystallographic studies of PCNA revealed two structurally symmetrical domains of the protein spanning P1 and P3 respectively, providing an explanation for p300 binding to both P1 and P3 regions of PCNA (51-53).

To confirm our in vitro observation, we analyzed the interactions in vivo utilizing co-immunoprecipitation (coIP) assays. HeLa cell nuclear extract was immunoprecipitated with control IgG, anti-p300 or anti-PCAF antibody. The immune complexes were immobilized onto protein G-sepharose beads, washed extensively, separated by SDS-PAGE and analyzed by immunoblotting using anti-p300, anti-PCAF or anti-PCNA antibody separately. Anti-p300 antibody immunoprecipitated PCNA as previously reported (Figure 3C, filled arrow). Yet, no PCNA was detected from the PCAF-immunoprecipitates under the same conditions (Figure 3C, open arrow). However, anti-PCAF antibody immunoprecipitated TAFII15 protein (a previously
known subunit of the PCAF complex) (Figure 3C), which therefore served as a positive control for anti-PCAF immunoprecipitation.

To further address whether PCNA selectively interacts with p300 but not PCAF in vivo, the sub-cellular localization of endogenous PCNA, p300 and PCAF was examined by immunohistochemical studies. PCNA was detected using anti-PCNA as the primary antibody and Cy3-conjugated donkey-anti-mouse as the secondary antibody (Figures 2D. a and d). p300 (Figure 2D.b) and PCAF (Figure 2D.e) were detected utilizing anti-p300 or anti-PCAF antibodies, respectively and FITC-conjugated goat-anti-rabbit secondary antibody. PCNA appeared to be nucleoplasmic and absent from the nucleoli in most of these asynchronized cells. In contrast to quiescent cells, actively growing cells constitutively express PCNA throughout the cell cycle but in late S phase, PCNA is also present in nucleoli (52). p300 was also localized throughout the nucleus (Figure 2D.b). PCAF displayed more limited staining in/around the nucleus (Figure 2D.e), which has been consistently observed by us using different anti-PCAF antibodies. The staining patterns of PCNA and p300 or PCAF were then merged and examined. Colocalization, shown as yellow, was observed for p300 and PCNA (Figure 2D.c). These results are also in agreement with those made by Hasan et al. demonstrating that the association between p300 and PCNA is cell cycle independent (35). However, little or no colocalization was observed for PCAF and PCNA (Figure 2D.f). These results were observed in both HeLa and NIH3T3 cells (data not shown), suggesting that specific association of p300 with PCNA and the lack thereof of PCAF and PCNA in vivo is a general phenomenon and not cell type specific. Together, these in vitro and in vivo results strongly indicate that PCNA associates with p300 but not PCAF.
PCNA selectively inhibits p300 dependent transcription when overexpressed in vivo

The HAT activity of p300 has been shown to play an important role in nuclear hormone receptor (NHR) signaling (16,54,55). We therefore utilized p300-dependent retinoic acid receptor (RAR)-mediated NHR signaling as a model system for the analysis of PCNA’s function in transcription in living mammalian cells. As previously reported, a GAL4-RARα fusion protein activated transcription of the reporter gene in a ligand, all trans retinoic acid (ATRA)- dependent manner (41). This ligand-dependent RAR signaling was inhibited in a dose dependent manner by an introduction of exogenous PCNA (Figure 3A). Next, we tested whether the direct transcriptional activation by a GAL4-p300 fusion would be similarly inhibited by transient transfection of PCNA in intact cells. GAL4-p300 activates transcription in a HAT activity dependent manner (11,41). In agreement with the results of the RAR-mediated transcriptional regulation, p300-mediated transcriptional activation was also blocked when PCNA was introduced (Figure 3B). In contrast, PCNA failed to show any inhibitory activity on GAL4-PCAF-mediated transcriptional activation (Figure 3C). Expression of exogenous PCNA was confirmed by immunoblot and it did not downregulate the expression of the GAL4 fusion proteins (data not shown). Therefore these results are consistent with the in vitro properties of PCNA and suggest a role for PCNA in selectively inhibiting p300 acetylase activity and its transcriptional activation properties.

PCNA directly represses transcription when targeted to a promoter

Since PCNA blocked activated transcription we next asked whether direct recruitment of PCNA to the promoter could mediate transcriptional repression. For that purpose, we fused PCNA to the C-terminal of Gal4-DBD and performed reporter gene assays in cells transfected
with gal4-responsive reporter and GAL4-PCNA. As observed previously and a control, GAL4-RAR repressed transcription of the reporter gene in the absence of the ligand ATRA (Compare Figure 4A lane 2 with lane 1). When ATRA was present, GAL4-RAR significantly activated the transcription of the reporter gene (Figure 4A, lane 3). The GAL4-PCNA fusion protein strongly repressed the transcription of the reporter gene (Figure 4A, lanes 4 and 5). In fact, as a GAL4-fusion, PCNA is more repressive than the well-studied transcriptional repressor, the unliganded RAR (compare lane 2 with lanes 4 and 5) in our assays. It was of interest to determine whether the GAL4-PCNA-P2 mutant (Figure 2B) is defective in repression. In contrast to GAL4-PCNA, GAL4-PCNA-P2, which lacks the p300 binding capability, failed to repress transcription of the reporter gene (Figure 4B). Western blot showed that both GAL4-PCNA and GAL4-PCNA-P2 were expressed at comparable levels (Figure 4C).

Chromatin plays an important role in gene regulation. Moreover, if PCNA repressed transcription, at least in part by regulating histone acetylation, the effect can best be studied with a chromosomally integrated reporter. Therefore, we also tested transcriptional regulatory roles of PCNA in 293T cells with chromosomally integrated gal4-TK-luc or gal4-SV40-luc promoter-reporter constructs. These integrated reporter cell lines were utilized to study transcriptional repression by thyroid hormone receptor (TR) (47). GAL4 or GAL4-PCNA plasmid was transfected into these cells and reporter activity measured. As observed before by others and us, transfection of increasing amounts of GAL4 plasmid caused a modest increase in transcription (Figures 4D and 4F, white bars). When GAL4-PCNA plasmid was introduced, a dose-dependent decrease in reporter activity from either gal4-TK (Figure 4D, black bars) or gal4-SV40 (Figure 4F, black bars) was observed. At the highest dose of plasmid, the reporter activity was reduced to greater than 50% of the control (un-transfected samples). The expression of GAL4 or GAL4-
PCNA in these cells was confirmed by performing immunoblot with the anti-GAL4 antibody using the same lysate used for the reporter assay (Figures 4E and 4G). These results were consistent with those obtained with the transiently transfected reporter, indicating that transiently transfected reporters also assume chromatin-like structure (56). Based on these results, we conclude that PCNA recruitment to either transfected or chromatin-linked promoters causes strong transcriptional repression.

**Targeting of PCNA to promoters causes chromatin hypoacetylation**

While hyperacetylation of histones promotes transcriptional activation, hypoacetylated histones are transcriptionally repressive (48). Since our data indicated that PCNA could inhibit HAT activity of p300 and GAL4-PCNA repressed transcription potently, it is possible that PCNA altered histone acetylation status of target promoters by decreasing histone acetylation. To explore this potential mechanism of repression by PCNA, we carried out chromatin immunoprecipitation (ChIP) assay, utilizing the same gal4-TK-luc and gal4-SV40-luc integrated reporter cell lines transfected with GAL4 or GAL4-PCNA plasmids. For each sample, immunoprecipitation was performed with control IgG, anti-acetyl-histone H3 or anti-acetyl-histone H4 antibody, as indicated. The precipitated DNA fragments were purified and amplified by PCR with primers specific to the TK (Figure 5A) or SV40 promoter (Figure 5B), respectively. For the PCR reaction, 2 different amounts of the same template were used to ensure the linear range of PCR reaction. Quantitation of the data from multiple experiments indicated that with the input DNA at comparable level from cells transfected with either GAL4 or GAL4-PCNA (lanes 1 and 2), a modest (30~35%) but reproducible decrease in histone H3 acetylation (lanes 4 and 5, compare upper and lower panel) and a more significant decrease (45~55%) in histone H4 acetylation (lanes 6 and 7, compare upper and lower panel) of promoters were
observed in the presence of GAL4-PCNA. This decrease in the acetylation level on the promoter did not result from a global decrease in histone acetylation when PCNA is overexpressed (Figure 5C). These in vivo results indicate that by promoting targeted hypoacetylation of histones/chromatin, PCNA could play a critical role in transcriptional repression of both activated and basal transcription.

From these results, we conclude that (a) PCNA inhibits the in vitro HAT activity of p300 but not of PCAF, (b) PCNA blocks p300 and RAR dependent transcriptional activation, but does not block PCAF-mediated transcription, (c) the selectivity of inhibition could be due to the differential binding of PCNA to p300 and PCAF, (d) PCNA can actively repress transcription when targeted to a promoter and (e) targeting of PCNA to a promoter causes hypoacetylation of histones/chromatin. Together, our results suggest that human PCNA may act as a selective inhibitor of p300-mediated histone acetylation and implicate a role for PCNA in transcriptional repression in vivo.

Discussion

Chromatin structure plays a fundamental role in the process of DNA replication, repair and transcription. Major components of these DNA-based processes were previously identified for their roles in individual pathways. For example, p300 was described as a component of the transcriptional regulatory pathway, while PCNA was shown to be an essential factor in DNA replication and repair. However, recent studies are now revealing broader and integrative roles for these and other regulatory factors in DNA-based processes (57,58). On one hand p300 has recently been implicated in PCNA-mediated DNA repair and synthesis (22,57). On the
other hand, a role for PCNA in transcription repression is implicated in many species. For example, in yeast PCNA mediates gene silencing although the mechanism is not known (32). In *Drosophila*, the PCNA mutant mus209B1 shows suppression of PEV in addition to its hypersensitivity to DNA damage. These results provided strong genetic proof for the role of *Drosophila* PCNA in transcriptional repression and heterochromatin maintenance (33). Again, the mechanism by which *Drosophila* PCNA may mediate repression was not established. In mammals, PCNA was recently demonstrated to associate with HDAC1 suggesting a role for histone deacetylation in DNA replication (34). However, neither a transcriptional regulatory role for human PCNA or the mechanism behind it has been explored.

In this report, we provide this first evidence that human PCNA functions as a potent regulator of transcription by associating with repressive state of chromatin. This is achieved by its ability to promote hypoacetylation status of histones and to mediate transcriptional repression. We demonstrate that PCNA is a selective HAT enzyme regulator in that, in vitro in the presence of HDAC inhibitor (10 mM sodium butyrate), recombinant PCNA could inhibit the HAT activity of p300, but not that of PCAF, indicating that this inhibition is specific. In agreement with the selectivity we observed, we show that PCNA associates with p300 and not PCAF both in vitro and in vivo. Moreover, our results indicate that the both N- and C- termini but not the central region of PCNA bind to p300. The physiological relevance of the effect of PCNA was further demonstrated in our reporter gene analysis indicating that PCNA specifically blocks p300 but not PCAF dependent transcription. While we have not exhaustively analyzed the regulation of all the processes individually mediated by p300 or PCAF, inhibition of retinoic acid receptor function implies that PCNA may serve as an in vivo regulator of p300-dependent pathways such as nuclear receptor signaling. Finally, association of PCNA with repressive status of chromatin was
established by the demonstration that chromatin targeted PCNA promotes hypoacetylation of histones and potent repression of the target genes in vivo.

The acetylation status of histones in chromatin is maintained by two opposing enzymatic activities, HATs and histone deacetylases. Therefore, it is conceivable that regulation of histone acetylation is necessary to achieve the correct “acetylation codes” that may be necessary for chromatin based processes including DNA repair, synthesis and transcription (48). In agreement with the above statement, our in vitro and in vivo results indicate that PCNA may play an important role in establishing the repressive histone code of transcription. Furthermore, our results suggest PCNA may establish repressive state of chromatin by multiple direct and indirect mechanisms. For example, PCNA by virtue of its ability to inhibit the HAT activity of coactivator(s) may protect nascently deacetylated histones and consequently help maintain hypoacetylation state of genes for repression. Alternatively, promoter-targeted PCNA may mediate repression by recruiting histone deacetylases and actively establishing hypoacetylation state of chromatin. Such a mechanism is consistent with the known interaction between PCNA and HDAC1 (34). We envision that both mechanisms either individually or in combination, may account for the potent repressive function of human PCNA reported here. Finally, our molecular and biochemical observations that human PCNA is a potent repressor of transcription, combined with previous genetic studies linking yeast and Drosophila PCNA to gene silencing strongly indicate that like its role in DNA synthesis and repair, PCNA also has a novel evolutionary conserved role in chromatin silencing. Our observation on transcriptional repression by human PCNA should allow future studies to determine the molecular components necessary for PCNA-mediated transcriptional repression.
Could the inhibition of the p300-acetyltransferase activity by PCNA also play any role in DNA repair and synthesis? As mentioned in the introduction, p300 is involved in PCNA-mediated DNA repair synthesis (35), implying that chromatin-remodeling activity of p300 may play a critical role in DNA repair and synthesis. Moreover, recent additional studies on DNA repair indicate that it may be necessary to regulate p300 acetyltransferase activity for proper DNA repair. Two important DNA repair factors, thymidine DNA glycosylase (TDG) and the endonuclease Fen1, can each serve as a substrate for p300 acetylase activity. TDG initiates repair at lesion sites at the excision step in mismatch repair. Recently, Tini et. al. demonstrated that p300 associates with TDG and suggested that such a recruitment of p300 may facilitate repair by promoting local chromatin remodeling. Interestingly TDG’s acetylation by p300 is proposed to inhibit the recruitment of the DNA-repair accessory factor, APE. Based on these observations the authors suggested that formation of a stable p300-TDG-APE complex at the repair sites might involve additional regulatory cellular factors either to initially inhibit the acetylase activity of p300 or to recruit histone deacetylase activity (57). In the case of Fen1, acetylation by p300 abrogates its endonuclease function necessary for DNA repair (36). Therefore it appears that maintenance of TDG or Fen1 in their unacetylated forms may be necessary at certain stages of DNA repair and that inhibition of acetyltransferase activity of recruited p300 by PCNA may serve that purpose to fulfill a general requirement for proper DNA repair. Alternatively, PCNA-suppression of histone/chromatin modification by p300 may also contribute directly to the DNA synthesis and repair processes.

Whether PCNA indeed blocks acetylation of TDG and/or Fen1 by p300 remains to be determined. However, based on our results that PCNA inhibits acetyltransferase activity of p300 by targeting the enzyme and not a specific substrate it is highly tempting to speculate that PCNA
may inhibit acetylation of TDG and/or Fen1. Because of its established role in DNA repair, PCNA will be an ideal candidate to provide the acetyltransferase inhibitory function necessary for stable p300-TDG-APE complex formation and maintenance of the endonuclease activity of Fen1 at the repair site. These hypothesis is also consistent with the previous observation that hyper-histone acetylation promoted by treatment of cells with HDAC inhibitor, sodium butyrate, negatively regulates DNA repair (59). However there are also reports suggesting hyperacetylation may enhance DNA repair (60). Future studies will be necessary to delineate the role of chromatin modification in DNA repair and synthesis.

In summary, our work presents the first evidence for the novel HAT-regulatory and transcriptional repressive function of human PCNA. Finally, our results also provide critical information to the evolving concept of integrative crosstalk among important chromatin-based processes such as transcription, epigenetic inheritance, DNA synthesis and repair.
Acknowledgements

We thank Drs. Y. Nakatani of Dana-Farber Cancer Institute, Boston, for providing anti-PCAF antibody, R.G. Roeder of Rockefeller University, New York for anti-TAFII15 antibody and M. Lazar for gal4-reporter integrated 293T cell lines. We also thank S. Kutney of our laboratory for providing purified histones and Dr. S.B. Seo previously of this laboratory for help during the initial stage of this work. We also thank Y. Mukai and F. Yelin for their technical help.

We thank Drs. B. Forman of Beckman Research Institute/Gonda Diabetes Research Center, the City of Hope National Medical Center, S. Ghosh of Yale University, New Haven, CT, I. Schuman of X-Ceptor Therapeutics, San Diego, and M. Tini of London Regional Cancer Center, Univ. of Western Ontario, Canada for critical reading of the manuscript.

Footnotes

This work was supported by National Institutes of Health Grant RO1-DK57079 (to D. C.) and NIDDK, National Institutes of Health Grant P30-DK50306 and the Abramson Cancer Center of the University of Pennsylvania, and funded, in part, under a grant with the Pennsylvania Department of Health. The Department specifically disclaims responsibility for any analyses, interpretations or conclusions.

Abbreviations

The abbreviations used are: HAT, histone acetyltransferase; HDAC, histone deacetylase; CREB, cAMP response element-binding protein; CBP, CREB-binding protein; PCNA, proliferating cell nuclear antigen; Fen1, flap endonuclease 1; INHAT, inhibitor of
acetyltransferases; NHR, nuclear hormone receptors; RAR, retinoic acid receptor; DBD, DNA binding domain; GST, glutathione S-transferase; PCAF, p300/CBP-associated factor; ATRA, all trans-retinoic acid; TDG, thymidine DNA glycosylase; PEV, position effect variegation.
References

1. Cheung, W. L., Briggs, S. D., and Allis, C. D. (2000) *Curr Opin Cell Biol* **12**, 326-333.

2. Mizzen, C. A., and Allis, C. D. (2000) *Science* **289**, 2290-2291.

3. Eckner, R., Ewen, M. E., Newsome, D., Gerdes, M., DeCaprio, J. A., Lawrence, J. B., and Livingston, D. M. (1994) *Genes Dev* **8**, 869-884.

4. Arany, Z., Sellers, W. R., Livingston, D. M., and Eckner, R. (1994) *Cell* **77**, 799-800.

5. Kwok, R. P., Lundblad, J. R., Chrivia, J. C., Richards, J. P., Bachinger, H. P., Brennan, R. G., Roberts, S. G., Green, M. R., and Goodman, R. H. (1994) *Nature* **370**, 223-226.

6. Hanstein, B., Eckner, R., DiRenzo, J., Halachmi, S., Liu, H., Searcy, B., Kurokawa, R., and Brown, M. (1996) *Proc Natl Acad Sci U S A* **93**, 11540-11545.

7. Yang, X. J., Ogryzko, V. V., Nishikawa, J., Howard, B. H., and Nakatani, Y. (1996) *Nature* **382**, 319-324.

8. Chen, H., Lin, R. J., Schiltz, R. L., Chakravarti, D., Nash, A., Nagy, L., Privalsky, M. L., Nakatani, Y., and Evans, R. M. (1997) *Cell* **90**, 569-580.

9. Cho, H., Orphanides, G., Sun, X., Yang, X. J., Ogryzko, V., Lees, E., Nakatani, Y., and Reinberg, D. (1998) *Mol Cell Biol* **18**, 5355-5363.

10. Ogryzko, V. V., Schiltz, R. L., Russanova, V., Howard, B. H., and Nakatani, Y. (1996) *Cell* **87**, 953-959.

11. Bannister, A. J., and Kouzarides, T. (1996) *Nature* **384**, 641-643.

12. Yao, T. P., Oh, S. P., Fuchs, M., Zhou, N. D., Ch'ng, L. E., Newsome, D., Bronson, R. T.,
Li, E., Livingston, D. M., and Eckner, R. (1998) Cell 93, 361-372.

13. Polesskaya, A., Naguibneva, I., Fritsch, L., Duquet, A., Ait-Si-Ali, S., Robin, P.,
Vervisch, A., Pritchard, L. L., Cole, P., and Harel-Bellan, A. (2001) Embo J 20, 6816-
6825.

14. Blanco, J. C., Minucci, S., Lu, J., Yang, X. J., Walker, K. K., Chen, H., Evans, R. M.,
Nakatani, Y., and Ozato, K. (1998) Genes Dev 12, 1638-1651.

15. Puri, P. L., Sartorelli, V., Yang, X. J., Hamamori, Y., Ogryzko, V. V., Howard, B. H.,
Kedes, L., Wang, J. Y., Graessmann, A., Nakatani, Y., and Levrero, M. (1997) Mol Cell
1, 35-45.

16. Korzus, E., Torchia, J., Rose, D. W., Xu, L., Kurokawa, R., McInerney, E. M., Mullen, T.
M., Glass, C. K., and Rosenfeld, M. G. (1998) Science 279, 703-707.

17. Warbrick, E. (2000) Bioessays 22, 997-1006.

18. Tsurimoto, T. (1998) Biochim Biophys Acta 1443, 23-39

19. Li, X., Li, J., Harrington, J., Lieber, M. R., and Burgers, P. M. (1995) J Biol Chem 270,
22109-22112.

20. Hosfield, D. J., Mol, C. D., Shen, B., and Tainer, J. A. (1998) Cell 95, 135-146.

21. Umar, A., Buermeyer, A. B., Simon, J. A., Thomas, D. C., Clark, A. B., Liskay, R. M.,
and Kunkel, T. A. (1996) Cell 87, 65-73.

22. Aboussekhra, A., Biggerstaff, M., Shivji, M. K., Vilpo, J. A., Moncollin, V., Podust, V.
N., Protic, M., Hubscher, U., Egly, J. M., and Wood, R. D. (1995) Cell 80, 859-868.

23. Shivji, M. K., Podust, V. N., Hubscher, U., and Wood, R. D. (1995) Biochemistry 34,
24. Chuang, L. S., Ian, H. I., Koh, T. W., Ng, H. H., Xu, G., and Li, B. F. (1997) Science 277, 1996-2000.

25. Shibahara, K., and Stillman, B. (1999) Cell 96, 575-585.

26. Xiong, Y., Zhang, H., and Beach, D. (1992) Cell 71, 505-514.

27. Hall, P. A., Kearsey, J. M., Coates, P. J., Norman, D. G., Warbrick, E., and Cox, L. S. (1995) Oncogene 10, 2427-2433.

28. Vairapandi, M., Balliet, A. G., Fornace, A. J., Jr., Hoffman, B., and Liebermann, D. A. (1996) Oncogene 12, 2579-2594.

29. Mathews, M. B., Bernstein, R. M., Franza, B. R., Jr., and Garrels, J. I. (1984) Nature 309, 374-376.

30. Elias, J. M. (1997) Biotech Histochem 72, 78-85.

31. Assy, N., and Minuk, G. Y. (1997) J Hepatol 26, 945-952.

32. Zhang, Z., Shibahara, K., and Stillman, B. (2000) Nature 408, 221-225.

33. Henderson, D. S., Banga, S. S., Grigliatti, T. A., and Boyd, J. B. (1994) Embo J 13, 1450-1459.

34. Milutinovic, S., Zhuang, Q., and Szyf, M. (2002) J Biol Chem 277, 20974-20978.

35. Hasan, S., Hassa, P. O., Imhof, R., and Hottiger, M. O. (2001) Nature 410, 387-391.

36. Hasan, S., Stucki, M., Hassa, P. O., Imhof, R., Gehrig, P., Hunziker, P., Hubscher, U., and Hottiger, M. O. (2001) Mol Cell 7, 1221-1231.
37. Chakravarti, D., Ogryzko, V., Kao, H. Y., Nash, A., Chen, H., Nakatani, Y., and Evans, R. M. (1999) *Cell* **96**, 393-403.

38. Hong, W., Kim, A. Y., Ky, S., Rakowski, C., Seo, S. B., Chakravarti, D., Atchison, M., and Blobel, G. A. (2002) *Mol Cell Biol* **22**, 3729-3743.

39. Ait-Si-Ali, S., Carlisi, D., Ramirez, S., Upegui-Gonzalez, L. C., Duquet, A., Robin, P., Rudkin, B., Harel-Bellan, A., and Trouche, D. (1999) *Biochem Biophys Res Commun* **262**, 157-162.

40. Zhang, Q., Vo, N., and Goodman, R. H. (2000) *Mol Cell Biol* **20**, 4970-4978.

41. Seo, S. B., McNamara, P., Heo, S., Turner, A., Lane, W. S., and Chakravarti, D. (2001) *Cell* **104**, 119-130.

42. Li, F., Macfarlan, T., Pittman, R. N., and Chakravarti, D. (2002) *J Biol Chem* **277**, 45004-45012.

43. Seo, S. B., Macfarlan, T., McNamara, P., Hong, R., Mukai, Y., Heo, S., and Chakravarti, D. (2002) *J Biol Chem* **277**, 14005-14010.

44. Chakravarti, D., LaMorte, V. J., Nelson, M. C., Nakajima, T., Schulman, I. G., Juguilon, H., Montminy, M., and Evans, R. M. (1996) *Nature* **383**, 99-103.

45. Kuo, M. H., and Allis, C. D. (1999) *Methods* **19**, 425-433.

46. Luo, R. X., Postigo, A. A., and Dean, D. C. (1998) *Cell* **92**, 463-473.

47. Ishizuka, T., and Lazar, M. A. (2003) *Mol Cell Biol* **23**, 5122-5131.

48. Strahl, B. D., and Allis, C. D. (2000) *Nature* **403**, 41-45.

49. Schiltz, R. L., Mizzen, C. A., Vassilev, A., Cook, R. G., Allis, C. D., and Nakatani, Y.
(1999) *J Biol Chem* **274**, 1189-1192.

50. Ogryzko, V. V., Kotani, T., Zhang, X., Schiltz, R. L., Howard, T., Yang, X. J., Howard, B. H., Qin, J., and Nakatani, Y. (1998) *Cell* **94**, 35-44.

51. Krishna, T. S., Kong, X. P., Gary, S., Burgers, P. M., and Kuriyan, J. (1994) *Cell* **79**, 1233-1243.

52. Kelman, Z. (1997) *Oncogene* **14**, 629-640.

53. Gulbis, J. M., Kelman, Z., Hurwitz, J., O'Donnell, M., and Kuriyan, J. (1996) *Cell* **87**, 297-306.

54. Kraus, W. L., Manning, E. T., and Kadonaga, J. T. (1999) *Mol Cell Biol* **19**, 8123-8135.

55. Perlmann, T., and Evans, R. M. (1997) *Cell* **90**, 391-397.

56. Reeves, R., Gorman, C. M., and Howard, B. (1985) *Nucleic Acids Res* **13**, 3599-3615.

57. Tini, M., Benecke, A., Um, S. J., Torchia, J., Evans, R. M., and Chambon, P. (2002) *Mol Cell* **9**, 265-277.

58. Chakravarti, D., and Hong, R. (2003) *Cell* **112**, 589-591.

59. Stoilov, L., Darroudi, F., Meschini, R., van der Schans, G., Mullenders, L. H., and Natarajan, A. T. (2000) *Int J Radiat Biol* **76**, 1485-1491.

60. Meijer, M., and Smerdon, M. J. (1999) *Bioessays* **21**, 596-603.
Figure legends:

**Figure 1:** HAT activity of p300, but not that of PCAF is inhibited by PCNA in a dose-dependent manner. (A) HAT assays were performed with increasing concentrations (0–0.6 µM) of purified GST-PCNA (lanes 5, 6, 7 and 8) or GST (0.6 µM) (lane 2). In lane 1, p300 was incubated with histones only. In lanes 3 and 4, p300 was incubated with increasing amounts of purified INHAT complex as positive controls. Positions of core histones H3, H2B, H2A and H4 are marked. (B) Similar assays as in (A) were performed and proteins were subjected to immunoblotting with anti-acetyl-H3 lysine14, anti-acetyl-H4 lysine 8 or anti-acetyl-H4 lysine 12 antibodies. (C) A similar HAT assay with PCAF was performed as described in (A) with histone H3 as a substrate. PCNA inhibits p300 but not PCAF-mediated histone acetylation.

**Figure 2:** PCNA binds to p300, but not PCAF both in vitro and in vivo. (A) PCNA binds to p300 but not histones in vitro. In vitro translated, [$^{35}$S]-labeled PCNA (lane 1) or pp32 (lane 2) was incubated with beads-bound Flag-p300 (lanes 3 and 4), Flag-PCAF (lanes 5 and 6) or core histones (lanes 7 and 8) as indicated. Bound proteins were separated by SDS-PAGE and analyzed by phosphorimager. As a positive control for histone binding, an INHAT subunit, pp32 binds strongly to core histones (lane 8). PCNA therefore binds to p300 but not PCAF or histones in vitro. (B) Multiple regions (P1 and P3) of PCNA interact with p300. Truncation mutants of PCNA: P1 (a.a 1-88), P2 (a.a. 89-178) and P3 (a.a. 179-261) were fused to GAL4-DBD. In vitro [$^{35}$S]-labeled GAL4 fusion proteins (marked by *) were incubated with beads-bound recombinant Flag-p300. After extensive wash, bound proteins were separated by SDS-PAGE and analyzed by phosphorimager. GAL4-DBD protein and GAL4-full length-PCNA were
included as controls. p300 binds to P1 and P3 but not P2. (C) Endogenous PCNA co-immunoprecipitates with p300 but not with PCAF. HeLa cell nuclear extract (600 µg) was immunoprecipitated with 4 µg of control IgG or anti-p300 antibody (α-p300) immobilized on protein G-Sepharose beads. After extensive wash, proteins in the immune complexes were separated by SDS-PAGE and subjected to immunoblotting with anti-p300 or anti-PCNA antibody as indicated. The filled arrow points to the presence of PCNA in p300 immunoprecipitates. A similar immunoprecipitation assay was performed with control IgG or anti-PCAF antibody (α-PCAF) followed by an immunoblotting with anti-TAFII15 (positive control) or anti-PCNA antibody as indicated. The open arrow points to the absence of PCNA in PCAF immunoprecipitates. (D) Endogenous PCNA colocalizes with p300 but not PCAF. (a-c) HeLa cells were subjected to double immunostaining with anti-PCNA (red, a) and anti-p300 (green, b) antibodies and analyzed by confocal microscopy. Merged a and b is shown in c. (d-f) HeLa cells were subjected to double immunostaining using anti-PCNA (red, d) and anti-PCAF (green, e) antibodies and analyzed by confocal microscopy. Merged d and e is shown in f. Inset shows the closer view of indicated cells. Endogenous PCNA associates with p300 but not PCAF.

**Figure 3:** PCNA blocks retinoic acid receptor (RAR) signaling and p300-dependent transcription. (A) PCNA inhibits ligand-dependent RAR transcriptional activation. NIH-3T3 cells were transiently transfected with the gal4- responsive reporter construct gal4-TK-luc, pCMX-GAL4-RAR and 0, 50, 100, 200 ng of pCMX-PCNA plasmid as indicated. 48 hours after transfection, cells were harvested and lysed for dual luciferase assay. (B) PCNA inhibits p300-dependent transcriptional activation. NIH3T3 cells were transfected with the reporter, GAL4-p300 and 0, 50, 100, 200 ng of pCMX-PCNA plasmid and subjected to reporter assays as
described in (A). (C) PCNA fails to inhibit PCAF-dependent transcriptional activation. NIH3T3 cells were transfected with the reporter, GAL4-PCAF and 0, 50, 100, 200 ng of pCMX-PCNA plasmid. Cell extracts were subjected to reporter assay as described in (A). PCNA blocks RAR and p300 but not PCAF-mediated transcription when overexpressed in cells.

**Figure 4:** GAL4-PCNA represses transcription of both transfected and chromosomally integrated reporter genes. (A) GAL4-PCNA represses transcription in transient transfection reporter assays. NIH3T3 cells were transfected with the reporter construct gal4-TK-luc alone (lane 1) or with 20ng of pCMX-GAL4-RAR in the absence (lane 2) or presence of ligand ATRA (lane 3) or 50 and 200 ng of GAL4-PCNA plasmids (lanes 4 and 5 respectively), then subjected to reporter assays as described in Figure 3(A). Note that the scale is broken to accommodate the activation profile of lane 3. (B) GAL4-PCNA-P2 fails to repress transcription in transient transfection reporter assays. 293T cells were transfected with the reporter construct gal4- TK-luc alone or with 100 and 200 ng of GAL4-PCNA or GAL4-PCNA-P2 plasmids, and subjected to reporter assays as described in Figure 3(A). (C) Cell lysate used in (B) was examined by Western blot with anti-GAL4 antibody. Both GAL4-PCNA and GAL4-PCNA-P2 were expressed in the cells at comparable levels. (D) 293T stable cell line with chromosomally integrated gal4-TK-luc reporter gene was transfected with 0, 100, 200 and 300 ng of GAL4-DBD or GAL4-PCNA plasmids, respectively, then subjected to reporter assays as described in (A). (E) Cell lysate used in (D) was examined by Western blot with anti-GAL4 antibody. Both GAL4 and GAL4-PCNA were expressed in the cells. (F) 293T stable cell line with chromosomally integrated gal4-SV40-luc reporter gene was transfected with 0, 100, 200 and 300 ng of GAL4-DBD or GAL4-PCNA plasmids, respectively, then subjected to reporter assays as described in (A). (G) Cell lysate used
in (F) was examined by Western blot with anti-GAL4 antibody, both GAL4 and GAL4-PCNA were expressed in the cells. PCNA potently represses transcription of transfected and chromosomally integrated reporter genes when targeted to a promoter as a GAL4 fusion protein.

**Figure 5**: Targeting of PCNA to chromatin causes a decrease in histone H3 and H4 acetylation. 293T cells with chromosomally integrated gal4-TK-luc (A) or gal4-SV40-luc (B) reporter gene were transfected with either GAL4-DBD or GAL4-PCNA plasmid. Following transfection, chromatin immunoprecipitation assays (ChIP) using either control IgG, anti-acetyl-histone H3 or anti-acetyl-histone H4 antibody were performed as described under Experimental Procedures. Increasing amounts of immunoprecipitated DNA fragments were amplified by PCR from the promoter region of the integrated gal4-TK-luc reporter gene (A) or gal4-SV40-luc reporter gene (B). (C) Overexpression of GAL4-PCNA did not change global acetylation of histones. 293T cells with chromosomally integrated gal4-TK-luc or gal4-SV40-luc reporter gene were transfected with increasing amount of GAL4-PCNA plasmid. Following transfection, cells were lysed and examined by Western blot with anti-acetylated histone H3 and anti-β-actin antibodies (loading control). Recruitment of PCNA to target chromatin domains only causes hypoacetylation of histones.
A. Histone acetylation by p300

B. Histone acetylation by p300

C. Histone acetylation by PCAF
A. gal4-TK-luc integrated 293T cells

|        | Input | IgG  | α-acetylated H3 | α-acetylated H4 |
|--------|-------|------|-----------------|-----------------|
| Gal4   |       |      |                 |                 |
| Gal4-PCNA |     |      |                 |                 |

B. gal4-SV40-luc integrated 293T cells

|        | Input | IgG  | α-acetylated H3 | α-acetylated H4 |
|--------|-------|------|-----------------|-----------------|
| Gal4   |       |      |                 |                 |
| Gal4-PCNA |     |      |                 |                 |

C. 293T:: gal4-TK-luc  293T:: gal4-SV40-luc

|        | Control | Gal4-PCNA | α-acetylated-H3 | α-β-actin |
|--------|----------|------------|-----------------|-----------|
|        |          |            |                 |           |
|        |          |            |                 |           |

Downloaded from http://www.jbc.org by guest on March 24, 2020
The human proliferating cell nuclear antigen, PCNA, regulates transcriptional coactivator p300 activity and promotes transcriptional repression
Rui Hong and Debabrata Chakravarti

J. Biol. Chem. published online August 23, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M303138200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts