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The SV40 T antigen modulates CBP histone acetyltransferase activity

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ABSTRACT

Histone acetyltransferases (HATs) play a key role in transcription control, cell proliferation and differentiation by modulating chromatin structure; however, little is known about their own regulation. Here we show that expression of the viral oncoprotein SV40 T antigen increases histone acetylation and global cellular HAT activities. In addition, it enhances CREB-binding protein HAT activity and modulates its transcriptional activity. Finally, we show that inhibition of cellular histone deacetylases by trichostatin A increases the SV40 infectivity rate. These findings highlight the importance of histone acetylation in the regulation of the cell cycle by oncoviral proteins.

INTRODUCTION

The packaging of the eukaryotic genome into chromatin provides the means for compaction of the entire genome inside the nucleus and restricts the access of many regulatory proteins required for essential biological processes such as replication, transcription, DNA repair and recombination (1). To counterbalance the repressive nature of chromatin, several factors are able to destabilise the nucleosome in order to facilitate the interaction of proteins with nucleosomal DNA. Recent studies have identified that there are two types of protein complexes capable of altering chromatin structure. One of them utilises the energy coming from ATP hydrolysis to alter the structure of nucleosomes (2). The second one includes complexes that covalently modify histones (3,4) by acetylation, methylation, phosphorylation, ubiquitination, etc.

Acetylation of the N-terminal tails of histones has been known for many years to be a process correlating with transcriptional activation (5,6), but the acetylation mechanisms and functions are just beginning to be revealed, thanks to the identification of the nuclear histone acetyltransferases (HATs). CBP/p300 is a coactivator protein (7), involved in both proliferative and differentiating pathways, which possesses HAT activity (8,9). CBP/p300 is ubiquitously expressed and regulates a broad spectrum of biological activities such as differentiation, cell cycle control, apoptosis and tumorigenesis. CBP/p300 is recruited to specific promoters through interactions with sequence-specific activators, and in turn recruits a second coactivator such as P/CAF (p300/CBP-associated factor) (10). These activators include CREB (11), c-Myb (12), MyoD (13), E2F1 (14), p53 (15) and nuclear hormone receptors (16). All of these interactions are critical for the execution of fundamental biological functions such as cell differentiation, cell proliferation, apoptosis, etc.

The mechanism of the CBP/p300-mediated activation of transcription is not clear, although we know that CBP/p300 contains multiple activation domains, it can contact the basal transcription factors TATA box-binding protein (TBP) and TFIIB (17), and its HAT domain is capable of activating transcription (18). Moreover, other coactivators that complex with CBP/p300, such as P/CAF, activator of retinoic acid receptor (ACTR) (19) and steroid receptor coactivator-1 (SRC-1) (20), themselves contain intrinsic nucleosomal HAT activity.

SV40 large T antigen is a multifunctional viral oncoprotein implicated in a wide range of cellular processes including transcriptional activation and repression, blockade of differentiation, stimulation of the cell cycle and cell transformation (21). The SV40 virus gains control of the cell through complex interactions between the viral oncoproteins and various intracellular proteins (22) involved in cell control and regulation of transcription, such as p53 (23,24), pRb and Rb-related proteins p107 and p130 (25–28), and CBP/p300 (29–32).

T antigen interacts with the p300 unphosphorylated but ubiquitinated form of the protein (30,31) and with CREB-binding protein (CBP) (30). In contrast to T antigen, the adenovirus early expression product E1A interacts with both phosphorylated and unphosphorylated forms of p300. Both T antigen and E1A affect transcription levels of the cAMP-responsive promoter that is modulated *in vivo* by p300 in REV2 cells. CBP/p300 also interacts with E1A protein and abrogates CBP/p300-mediated transcriptional activity (33). The mechanism by which this repression occurs is not yet clear. It could occur by sequestration of CBP/p300 away from the promoter; alternatively, E1A may displace the CBP/p300-associated factor, which is required for efficient activation of transcription. Another possibility (34,35) it is that E1A inhibits the HAT activity of CBP/p300 and P/CAF.

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However, another study has shown an activation of CBP HAT activity by E1A (36).

Since T antigen also interacts with CBP (30) but very little is known about the consequences on CBP activity, we asked whether T antigen is able to modulate the HAT activity of host cells as well as the CBP coactivator. We show that T antigen expression increases global histone acetylation and CBP HAT activity. Moreover, the T antigen can stimulate HAT-mediated CBP transcriptional activity in a Gal4-hsp70 promoter. Finally, we show that inhibition of cellular histone deacetylases (HDACs) increases the SV40 infectivity rate.

MATERIALS AND METHODS

Constructs

pSG5-T antigen plasmid was a gift of Dr J. DeCaprio. pSG5-T antigen ΔCBP contains a 379 bp deletion. It was made by partial digestion of the pSG5-T antigen plasmid with NsiI restriction endonuclease, posterior digestion of the purified 6.3 kb fragment with PstI restriction endonuclease and religation of the purified 5.9 kb fragment. pCDNA3Gal4-HAT, pCDNA3Gal4-HAT-CBP2 and pCDNA3Gal4(DBD) constructs have been described elsewhere (18) and were kindly provided by Dr T. Kouzarides. The Gal4-hsp70-β-gal reporter was supplied by Dr J. Bernues. The pCMV-DP1 construct has been described elsewhere (14) and was kindly provided by Dr D. Trouche.

Cell culture, transfections and reporter gene assay

CV1 and CV1COS cells were maintained in Dulbecco's modified Eagle's medium (DMEM) with 10% foetal calf serum (FCS) and grown at 37°C, 5% CO_2. G_1 cell synchronisation was achieved by removal of FCS from the medium (DMEM) for 36 h and collection of cells 8 h after addition of FCS to the medium.

Cells were transfected by a standard calcium phosphate co-precipitation (for β-galactosidase assays) or using a Jet-PEI Kit (GenyCell) (for HAT assays; by using this method, ~80% of the cells were transfected, as we determined by fluorescence after co-transfection with pCDNA3-GFP) with 0.1–2 μg (as indicated) of either expression vector and 2 μg of β-galactosidase reporter plasmid. The cells were washed after 14 h and harvested 36 h after transfection. After that, they were washed once in phosphate-buffered saline (PBS). For β-galactosidase assay, cells were lysed in 150 μl of β-galactosidase lysis buffer (Promega) for 20 min at room temperature. Supernatants were clarified by centrifugation (2 min, 12 000 *g*) and 30 μl were mixed with 270 μl of β-galactosidase assay solution (Promega) and analysed at OD_{420}.

Cell extract preparation, histone extraction and estimation of the protein content

Histones were extracted by overnight treatment of whole cells with 0.25 M HCl at 4°C under rotation. After centrifugation at 12 000 *g* for 10 min at 4°C, histones were precipitated by cold acetone and resuspended in SDS-protein loading buffer. Total cell extracts were prepared as follows: cells were lysed in 1 ml of buffer A (50 mM Tris–HCl, pH 8, 150 mM NaCl, 0.5% NP-40, 10 μg/ml of aprotinin and leupeptin, 1 mM phenylmethylsulfonyl fluoride, 50 mM NaF and 10 mM Na_2-β-glycerophosphate) by keeping the cells on ice for 20 min, and cell debris was removed by centrifugation at 12 000 *g* for 10 min at 4°C. Estimation of the protein content was done by using the 'Bio-Rad protein Assay reagent' following the the manufacturer's instructions.

Immunoprecipitations and western analysis

Immunoprecipitations and western blot analysis were performed as described elsewhere (37) with modifications: 10-cm dishes of CV1 cells and transfected CV1 cells with the indicated amount of each expression vector were harvested 36 h post-transfection. Cells were lysed in 1 ml of buffer A by keeping them on ice for 20 min, debris was removed by centrifugation and the cleared lysate was subjected to immunoprecipitation with 2 μg of CBP antibody for 3 h at 4°C with rotation. A 15 μl aliquot of protein A/protein G–Sepharose beads (a 50:50 mix) was then added and incubated overnight at 4°C under rotation. The immune complexes were pelleted by gentle centrifugation and washed three times with 1 ml of lysis buffer B (20 mM Tris–HCl, pH 7.5, 100 mM NaCl, 1 mM EDTA and 0.5% NP-40). After the final wash, the buffer was aspirated and the antibody beads were either resuspended in loading buffer for SDS–PAGE to be analysed by western blot or used in the HAT assay as described below. Western blotting was performed using standard procedures and visualised using an enhanced chemiluminescence kit (Amersham). The antibodies used were: whole antiserum anti-T antigen (a gift from Dr J. DiCaprio), affinity-purified anti-CBP, A22 (Santa Cruz Biotechnology) at 1:1000 dilution, affinity-purified anti-hemagglutinin (HA; Upstate Biotechnology) at 1:1500 dilution, and whole antiserum anti-acetyl-lysine, antibody 193 (abcam) at 1:100 dilution; this antibody was raised against chemically acetylated human histone H4 and reacts with ε-acetylated lysines of the four core histones.

HAT and IP-HAT assays

IP-HAT assays were performed as described elsewhere (8,18). Basically, extracts from CV1, CV1COS or transfected CV1 cells were made using 500 μl of buffer A. The lysis mixture was incubated on ice for 20 min and cleared by centrifugation at 12 000 *g* for 10 min at 4°C. A 2 μg aliquot of CBP antibody was used to precipitate the CBP for 3 h at 4°C with rotation, then 15 μl of protein A/protein G–Sepharose beads (a 50:50 mix) were added and incubated overnight at 4°C under rotation. The immune complexes were pelleted by gentle centrifugation and washed three times with 1 ml of buffer B. After the final wash, the buffer was aspirated down to 30 μl and the liquid HAT assay was done as described (8). Purified chicken erythrocyte histones (Sigma) were use as substrate.

Viral infection

Subconfluent CV1 cells were infected with the SV40 virus as described in Su and DePamphilis (38), and grown for 8–10 days in DMEM with 5% FCS, 0.9% agar and 75, 100 or 150 nM Trichostatin A (TSA). After 8–10 days, they were stained with neutral red and the lysis colonies were counted after 24–48 h.

Bioinformatics tools

Sequence searches were done using the PSI-BLAST program (39) with default parameters. The multiple sequence alignment for the E1A family was obtained from the Pfam database (40), with accession number PF02703.

RESULTS

T antigen expression affects cellular histone acetylation

Several independent findings indicate that HATs and HDACs play an important role in cell cycle regulation, suggesting that these enzymes represent likely targets of viral oncoproteins that deregulate the cell cycle.

To test whether global HAT activities are modified by the SV40 regulatory oncoprotein T antigen, we analysed the HAT activity of cells expressing (CV1COS cell line) and not expressing (CV1 cell line) the SV40 T antigen, by an *in vitro* HAT assay. To that end, total cellular extracts from CV1 and CV1COS were prepared, the total protein amount was adjusted and the extracts were incubated with purified chicken erythrocyte core histones and [^{3}H]acetyl-CoA. The level of histone acetylation was analysed by fluorography (Fig. 1A) and quantified in a scintillation counter (Fig. 1B). The global HAT activity was ~2.3 fold (1.9–2.5) higher in T antigen-expressing cells (Fig. 1B). To confirm this result, we transfected the T antigen on CV1 cells (80% of the cells were transfected), adjusted the total protein amount in the cell extracts and determined the HAT activity of T antigen-transfected and non-transfected cells by liquid HAT assay. The results in Figure 1C show an increased global HAT activity after T antigen expression.

We then aimed at elucidating whether the histones from cells expressing the T antigen were more acetylated than those from non-expressing cells. To this end, histones from both CV1 and CV1COS cells were prepared by acid extraction and their level of acetylation was analysed by western blot using an antibody raised against chemically acetylated human histone H4 which reacts with ε-acetylated lysines of the four core histones (abcam, ab 193) (41). The results in Figure 2 show that the acetylation level of H3, H2A/B and H4 in T antigen-expressing cells (CV1COS) was 5-fold higher than in non-expressing cells (CV1). The acetylation levels of H3 and H2A/B increased more than H4. In particular, the acetylation of H2A/B was as high as H4 acetylation, suggesting that T antigen expression could lead to acetylation of all lysines on H2A/B histones tails *in vivo*. One reason for such a high H2A/B acetylation could be the fast DNA replication after viral infection. Alternatively, changes in specificity of cellular HAT after viral infection could account for the observed histone acetylation pattern change. According to that, it would be interesting to check whether the same increase in H2A/B acetylation occurs after infection by other DNA viruses.

These results support that T antigen expression leads to a global increase of HAT activities and histone acetylation level.

T antigen expression affects CBP HAT activity

Given that T antigen binds the third finger on the CBP molecule, very close to the HAT domain, and that T antigen expression increases cellular histone acetylation (see above),

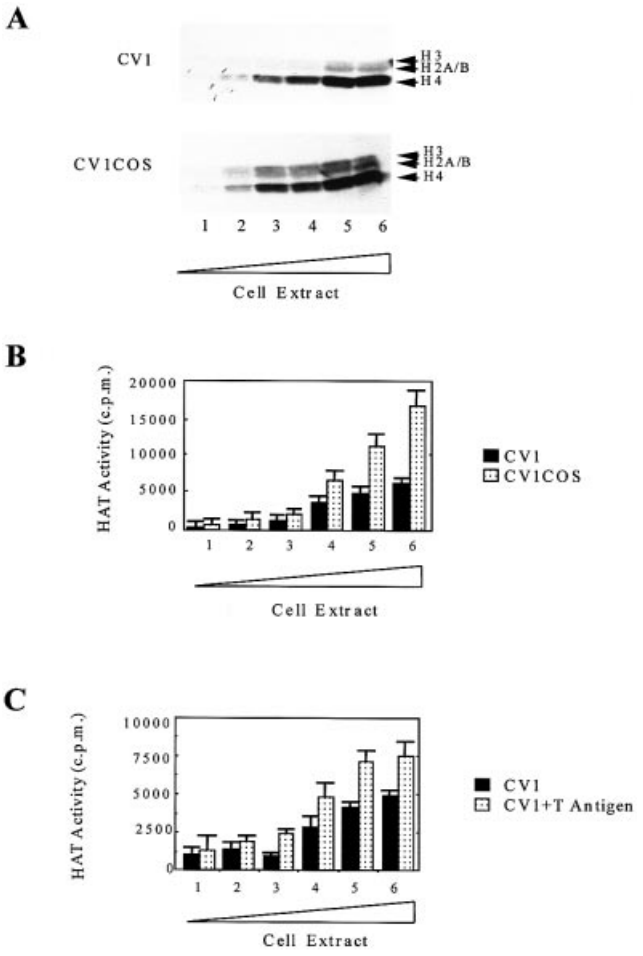

Figure 1. Expression of T antigen increases cellular global HAT activity. (**A**) Increasing amounts (0.1–10 µg) of CV1 and CV1COS whole-cell extracts were tested for their ability to acetylate free histones in an *in vitro* HAT assay (8). Basically, whole-cell extracts were incubated with purified chicken erythrocyte histones (Sigma) and [^{14}C]acetyl-CoA at 30°C for 30 min. The level of histone acetylation was analysed in an 18% polyacrylamide gel and revealed by fluorography. (**B**) To quantify the HAT activity present in CV1 and CV1COS cells, increasing amounts (0.1–10 µg) of CV1 and CV1COS whole-cell extracts were tested for their ability to acetylate free histones in a standard liquid HAT assay. HAT activity is expressed as radioactivity (c.p.m.) associated with ^{3}H-labelled acetylated core histones. (**C**) CV1 cells were transfected with 10 µg of pSG5-T antigen and pCDNA3-GFP using a Jet-PEI Kit (GenyCell). Around 80% of the cells were transfected, as determined by fluorescence. Whole-cell extracts from transfected and non-transfected cells were prepared and used in a liquid HAT assay as in (B). The global HAT activity associated with cells not expressing and expressing T antigen is expressed as radioactivity (c.p.m.) associated with ^{3}H-labelled acetylated core histones.

we next determined whether T antigen expression affects the intrinsic CBP HAT activity. Using an IP-HAT assay approach, CBP from cells expressing or not expressing the T antigen was immunoprecipitated and the HAT activity within the immunopellet assessed. Figure 3A shows that CBP HAT activity in cells expressing T antigen was higher (2.3-fold increase) than in cells lacking the T antigen, especially in cells arrested in G_1 by serum starvation (4.2-fold increase) (Fig. 3B). Similar amounts of CBP were immunoprecipitated in CV1 and CV1COS cells, as shown in the bottom panels of Figure 3A

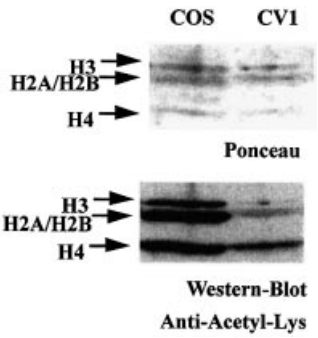

Figure 2. Total histones were prepared by acid extraction from CV1 and CV1COS cells and their acetylation levels were analysed by western blot using an antibody that recognises acetylated lysines (ab 193; abcam) (bottom panel). The top panel shows the Ponceau staining of the gel as a histones level control. Histones (H3, H2A/B and H4) are indicated by arrows.

and B. Western blot analysis revealed no differences in the amount of CBP in the two cell lines (Fig. 3C). To investigate whether T antigen was directly responsible for this increase in CBP HAT activity *in vivo*, we introduced a deletion at the C-terminal CBP-binding region on the T antigen (amino acids 401–536) protein which rendered the T antigen unable to bind CBP (Fig. 3D, bottom panel); it was named ΔCBP. We then transfected the wild-type T antigen and the mutant (ΔCBP) T antigen on CV1 cells (~80% of the cells were transfected), and afterwards we prepared whole-cell extracts, adjusted the total protein amount in transfected and non-transfected cell extracts and determined CBP HAT activity, as previously described. CBP HAT activity showed a reproducible increase after expression of the wild-type T antigen (Fig. 3D) but not of the ΔCBP T antigen. The expression level of wild-type and ΔCBP T antigen after transfection on CV1 cells was similar (Fig. 3D, top panel). The confirmation of this result *in vitro*, by using recombinant purified proteins, was not possible due to the low level of T antigen expression in *Escherichia coli*.

Altogether, these data suggest that the oncoprotein T antigen modulates cellular HAT activities and in particular CBP HAT activity.

Given that T antigen affects CBP HAT activity, we next tested whether the CBP specificity for core histones is altered by T antigen expression. To do this, CBP from CV1 and CV1COS cells was immunoprecipitated using antibodies against CBP and HA (as a control antibody) and the HAT activity associated with the immunoprecipitates was analysed by fluorography (Fig. 4A), quantified by densitometry in a Molecular Dynamics machine (Fig. 4B, top panel) and normalised relative to the value in CV1 cells (value: 1) (Fig. 4B, bottom panel). The results in Figure 4A and B confirm an increased CBP HAT activity on CV1COS cells and show no modification of the CBP specificity for core histones by the T antigen.

T antigen modulates CBP HAT-mediated activation

Since T antigen affects CBP HAT activity, we aimed at determining whether it also modulates the transcriptional activity mediated by the CBP HAT domain. To avoid interference by other CBP domains, in particular the C-terminus (capable of binding to other proteins such as P/CAF and SRC-1, which also carry their own HAT activity), we separated the CBP HAT and CBP2 (the CBP third finger, where the T antigen binds CBP) domains (HAT-CBP2) from the rest of the molecule and fused them with the Gal4 DNA-binding domain (Gal4-HAT-CBP2). Previous results (18) have shown that a region of CBP, encompassing the previously defined HAT domain, can stimulate transcription when tethered to a promoter. This stimulatory effect is dependent on HAT activity. The activation capacity of the CBP HAT domain increases by the fusion of the CBP2 domain (which harbours the binding site for a variety of regulatory transcription factors: E2F and c-Fos).

First, we measured the transcriptional activity mediated by the Gal4-HAT-CBP2 domains on the hsp70 promoter, which contain five Gal4-binding sites, by transient transfection, and then we examined whether wild-type and ΔCBP T antigen affect the transcriptional activity of the Gal4-HAT-CBP2. The results in Figure 5 show that Gal4-HAT-CBP2 activates the hsp70 promoter. We also observed that T antigen efficiently stimulated the ability of HAT-CBP2 to activate the hsp70 promoter (Fig. 5A) in a specific manner because, when expressing another protein (DP1), no effect was found (Fig. 5A). This T antigen coactivation is dependent on CBP binding because the mutant T antigen (ΔCBP) was also defective in coactivation (Fig. 5B). To confirm this result, we have also used a CBP construct that contains only the HAT domain and lacks the CBP2 region (T antigen-binding region) fused to the Gal4 DNA-binding domain (Gal4-HAT). This construct is still able to activate transcription, but is a very weak activator (18). The results in Figure 5C show that the coactivating effects of T antigen on the transcriptional activity CBP HAT (which has lost the T antigen-binding site) are substantially reduced.

Altogether, the results from the transfection experiments strongly suggest that T antigen binding to CBP (through the CBP2 region) modulates the transcriptional activity mediated by the CBP HAT domain on the hsp70 promoter.

The histone deacetylase inhibitor, TSA, increases the SV40 infectivity rate

Our previous results show that expression of the T antigen leads to an increase in cellular HAT activity, suggesting that T antigen oncoprotein targets HATs and HDACs to deregulate the cell cycle and/or to regulate viral and cellular genes. We next tested whether inhibition of HDAC activity could affect viral growth and infectivity. CV1 cells were infected with SV40 and maintained in the presence or absence of increasing nanomolar concentrations of the potent HDAC inhibitor TSA. After 7–10 days, the cells from duplicate dishes were stained with neutral red and viral lysis plates were counted. In the presence of TSA, the growth rate of SV40 was clearly higher and more lysis plates were generated (3- to 6.5-fold) than in the absence of the HDAC inhibitor (Table 1). In addition, after TSA treatment, lysis plates appeared earlier (2–4 days) than in non-treated cells. Similar growth rates were observed with 75–100 nM TSA (above this concentration, cell death started to appear) and at various infection multiplicities (from 4 to 40 p.f.u.).

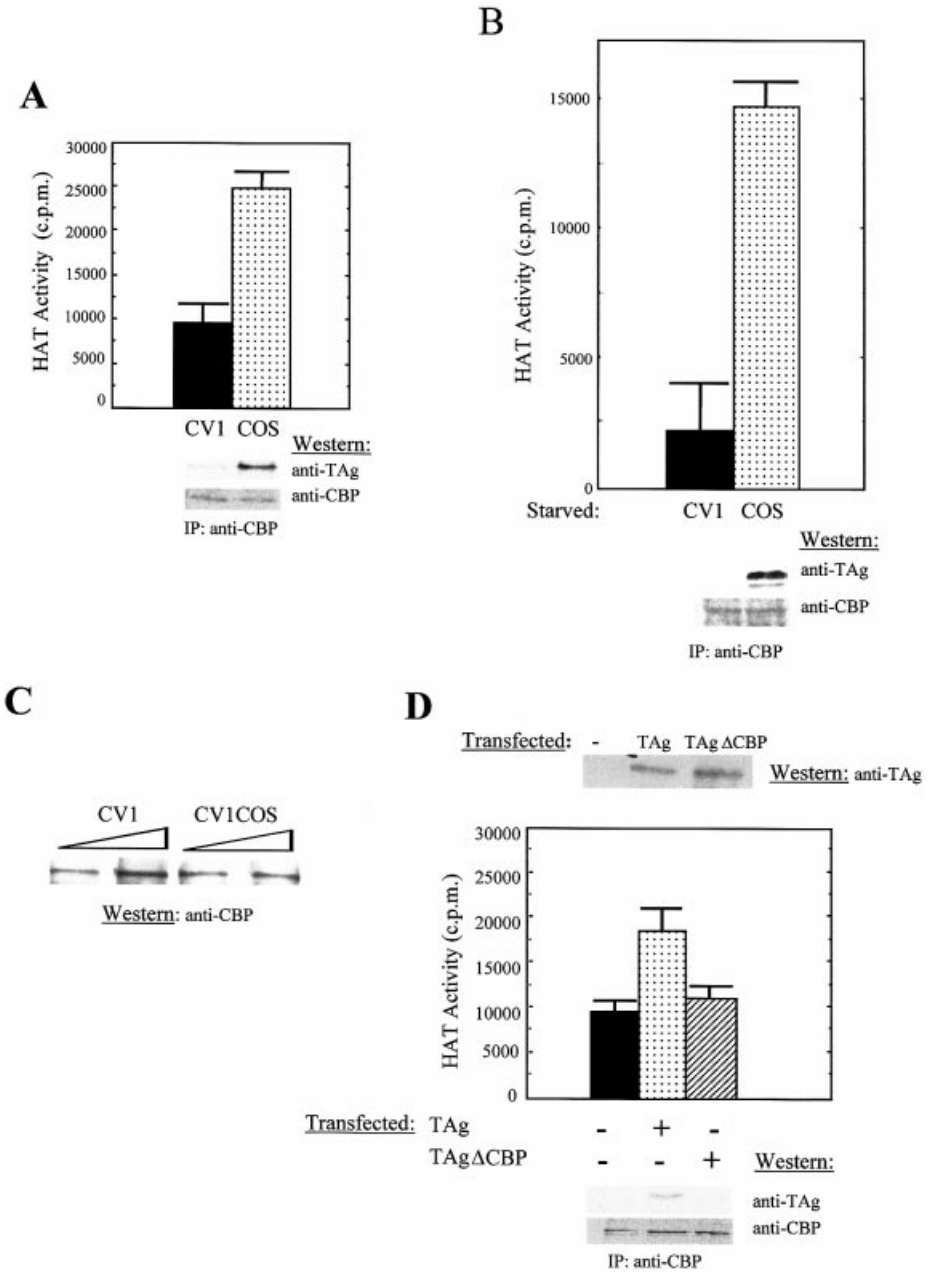

Figure 3. CBP HAT activity increased after T antigen expression. (**A**) An equal amount of total protein of CV1 and CV1COS whole-cell extracts was precipitated with anti-CBP antibody and the immunocomplexes were tested for their ability to acetylate free histones in a liquid HAT assay. The presence of T antigen and CBP in the immunoprecipitates in CV1 and CV1COS cells was tested by western blot using anti-T antigen and anti-CBP antibodies, respectively (shown in the bottom part). The data are the average of at least four independent experiments. (**B**) CV1 and CV1COS cells were serum starved, and the HAT activity analysis was performed as in (A). (**C**) Increasing amounts of CV1 and CV1COS whole-cell extracts were loaded onto a 7% polyacrylamide gel, and the amounts of CBP in the two cells lines were determined by western blot using anti-CBP antibody. (**D**) CV1 cells were transfected with 10 µg of pSG5-T antigen (TAg), pSG5-T antigenΔCBP (TAgΔCBP) antigen and pCDNA3-GFP using a Jet-PEI Kit (GenyCell). Around 80% of the cells were transfected by this method, as confirmed by fluorescence. Whole-cell extracts from transfected and non-transfected cells were prepared. An equal amount of total protein was precipitated with anti-CBP antibody and the immunocomplexes tested for their ability to acetylate free histones in a liquid HAT assay (top part) and for the presence of CBP and T antigen by western blot using anti-CBP and anti-T antigen antibodies, respectively (bottom part). The expression levels of TAg and TAgΔCBP after transfection were determined by western blot using anti-T antigen antibody (top part). The data are the average of at least three independent transfections.

DISCUSSION

The SV40 virus requires cellular proliferation for its own replication. Therefore, one of its regulatory proteins, the T antigen, has evolved to steer cells through the cell cycle. To transform mammalian cells, T antigen needs to interact with three different proteins: one that includes proteins from the Rb-pocket family (resulting in the loss of the ability to suppress growth), p53 and proteins from the CBP/p300 family of coactivators (which facilitate cellular transformation). Here

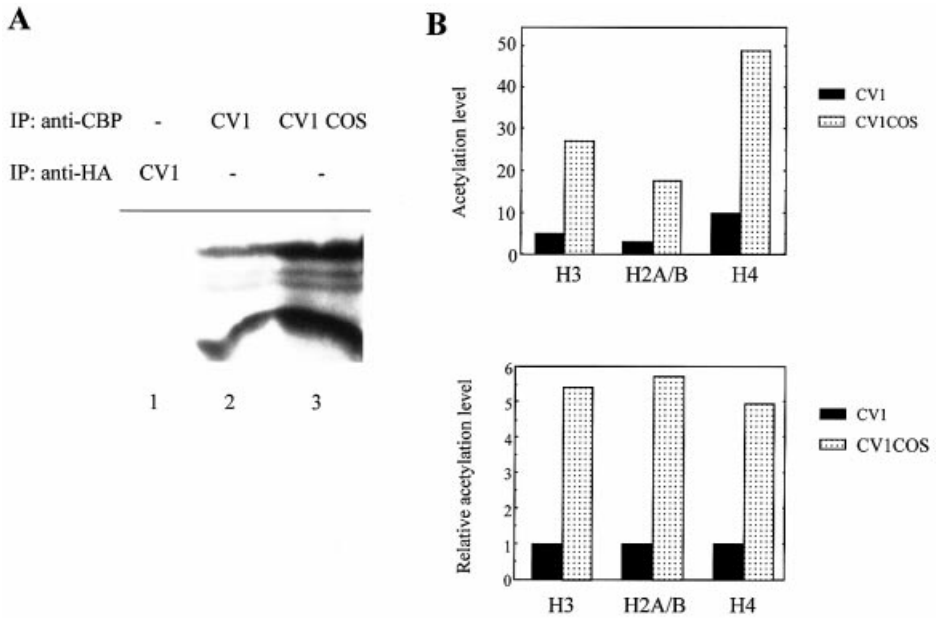

Figure 4. CBP specificity for core histone does not change after T antigen expression. An equal amount of total protein of CV1 and CV1COS whole-cell extracts was precipitated with anti-CBP and HA antibodies and the immunocomplexes tested for their ability to acetylate free histones in an *in vitro* HAT assay. (**A**) Fluorograph showing the HAT activity of CBP from CV1 and CV1COS cells. (**B**) The bands corresponding to H3, H2A/B and H4 histones were quantified by densitometry using a Molecular Dynamics machine (top panel). The acetylation levels of H3, H2A/B and H4 in CV1COS cells were normalised relative to the acetylation levels in CV1 cells (value 1) (bottom panel). The acetylation level of the four core histones increased in CV1COS cells between 5- and 6-fold relative to the acetylation level of CV1 cells.

we have shown that the interaction of T antigen with CBP modulates CBP activities. T antigen expression increases CBP HAT activity, which is higher when the cells have been serum starved. As reported elsewhere, CBP HAT activity is modulated by another viral protein, E1A, although there is considerable controversy in the literature: Ait-Si-Ali *et al.* (36) have shown that E1A increases CBP HAT activity to a level similar to that obtained in this study, whereas Chakravarti *et al.* (35) have proposed that CBP and P/CAF HAT activities are inhibited through interaction with E1A, as confirmed by Hamamori (34). On the other hand, work from Kouzarides' lab shows that E1A does not alter P/CAF HAT activity *in vitro* or *in vivo* (42). In the case of T antigen, ours is the first report showing a modulation of CBP HAT activity, which supports the hypothesis that cellular HAT activities are a direct target for acetyltransferase regulation by viral oncoproteins.

We have also shown that global histone acetylation status increased after T antigen expression. We believe that this global increase is mainly a consequence of the higher global HAT activity (as shown in Fig. 1), or due to the regulation of HAT accessibility to substrate. However, we cannot exclude some indirect effects due to the T antigen-induced transformation process.

What could be the meaning of a global increase in the histone acetylation level? Treissman *et al.* (43) have shown that H4 hyperacetylation can occur even when transcription is blocked by actinomycin D, suggesting that global histone hyperacetylation can occur when transcription is not active. Transcriptional HATs also play an apparent non-transcriptional role in establishing the total genomic balance of acetylation (44–46). Global acetylation may thus represent a general response of the cells to a stimulus, in this case a viral oncoprotein, which may alter the threshold level to trigger transcription. On the other hand, the general increased histone acetylation level may be due merely to the addition effects of the locally higher acetylation from T antigen-regulated promoters.

E1A and other CBP/p300-binding proteins may interact with the CBP TRAM motif through the FPESDLIL sequence (47). We searched the SV40 T antigen for this motif by sequence comparison. FPESLIL aligned with a similar peptide in the SV40 T antigen. However, the match falls in an SV40 T antigen region that does not bind CBP. In addition, the BLAST program (48) provides even better identities for the FPESDLIL sequence to proteins that have not been described as CBP partners and show very divergent functions. These two facts suggest that the observed match in the SV40 T antigen is fortuitous. By comparing the whole E1A and SV40 T antigen sequences, we found a certain percentage of identity between two sequence stretches, which do not correspond to the binding site of any of the proteins. Finally, we looked for the pattern FX (D, E) XXXL, identified by E1A and other third finger CBP-interacting proteins (p53 and E2F), in the SV40 T antigen, but did not find it. This may be due to the fact that the E1A pattern was derived from a reduced set of protein sequences. When more sequences become available, the pattern may not be as conserved as it seemed from the original data. In the Pfam (39) alignment for the E1A family of proteins, there are changes in all the pattern positions (Fig. 6), even in those that had been considered fixed. The SV40 T antigen and E1A could thus have a structurally similar binding surface to CBP, but corresponding to a degenerate sequence pattern. A second option could be that the SV40 T antigen has

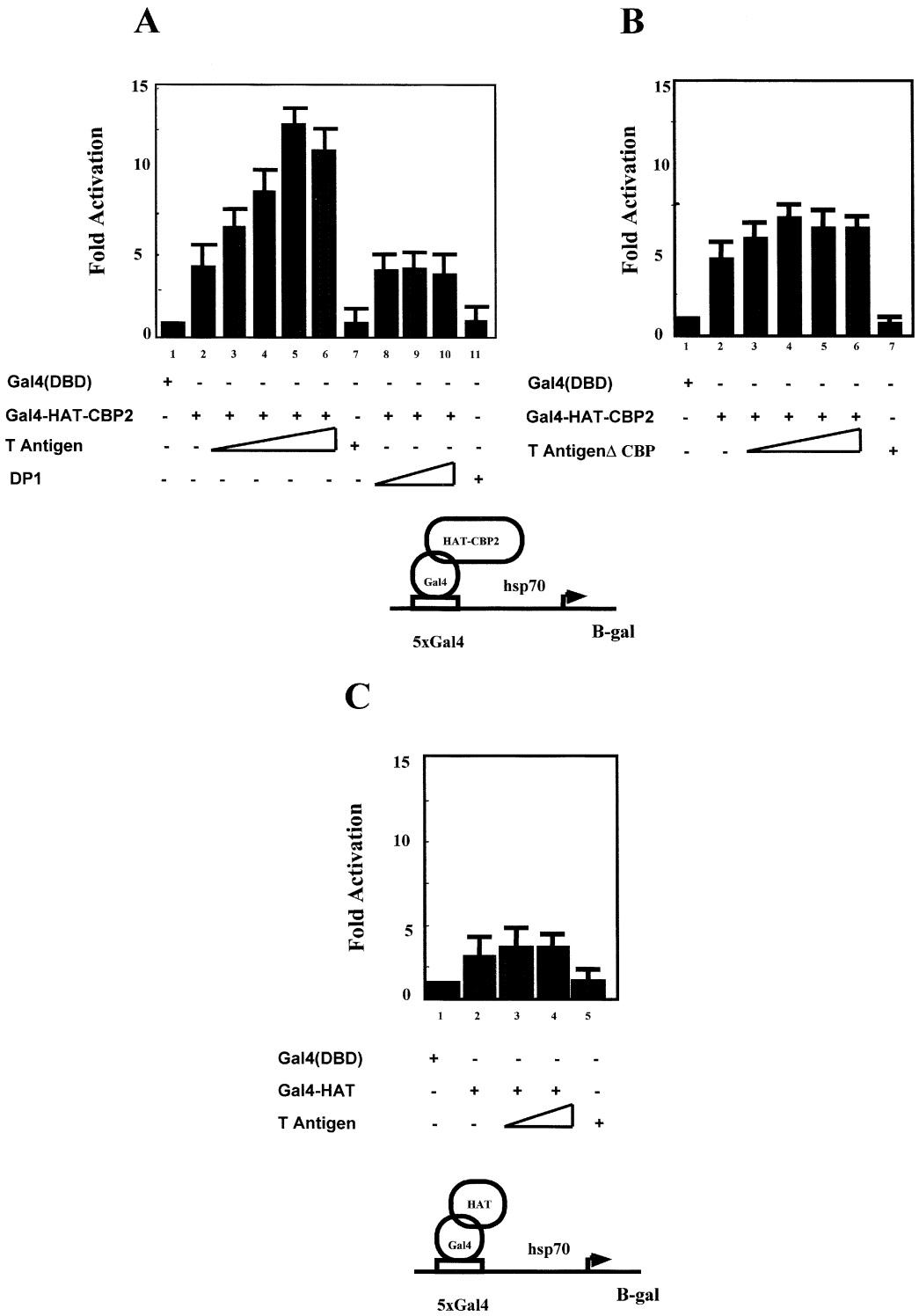

Figure 5. T antigen activates CBP HAT-mediated activation on the hsp70 promoter. (**A**) CV1 cells were transfected with 2 μg of the Gal4-hsp70-β-gal reporter, 2 μg of Gal4 DNA-binding domain (DBD) or Gal4-HAT-CBP2, and increasing amounts (0.1–2 μg) of pSG5-T antigen, pCDNA3-DP1 or the empty vector. Whole-cell extracts were used in the β-galactosidase assay. The activity derived from the Gal4-hsp70-β-gal reporter plus the pcDNA3-Gal4(DBD) was normalised to 1.0, and the other activities are expressed relative to this. The data are an average of at least six independent transfections. (**B**) T antigen activates CBP HAT-mediated activation in a CBP binding-dependent manner. CV1 cells were transfected with 2 μg of the Gal4-hsp70-β-gal reporter, 2 μg of Gal4 DBD or Gal4-HAT-CBP2, and increasing amounts (0.1–2 μg) of pSG5-T antigen ΔCBP or the empty vector. Whole-cell extracts were used in the β-galactosidase assay. The activity derived from the Gal4-hsp70-β-gal reporter plus the pCDNA3-Gal4(DBD) was normalised to 1.0, and the other activities are expressed relative to this. The data are an average of at least four independent transfections. (**C**) CV1 cells were transfected with 2 μg of the Gal4-hsp70-β-gal reporter, 2 μg of Gal4 DBD or Gal4-HAT, and 0.5 and 2 μg of pSG5-T antigen or the empty vector. The transcriptional activity of the hsp70 promoter was determined as above. The data are an average of at least three independent transfections.

Table 1. Increased growth rate of SV40 virus in the presence of TSA

P.f.u.	TSA (nM)		
	0	75	100
0	1	1	1
4	1	6.5	5
20	1	5.6	3
40	1	3	–

CV1 cells were infected with SV40 virus as described in Su and DePamphilis (38), and they were grown for 8–10 days in DMEM with 5% FCS, 0.9% agar and 0, 75 and 100 nM TSA. After 8–10 days, the cells were stained with neutral red and the lysis colonies were counted after 24–48 h. The numbers of colonies are normalised in non-treated cells to 1. Data are the average lysis plates from duplicate dishes from three independent experiments.

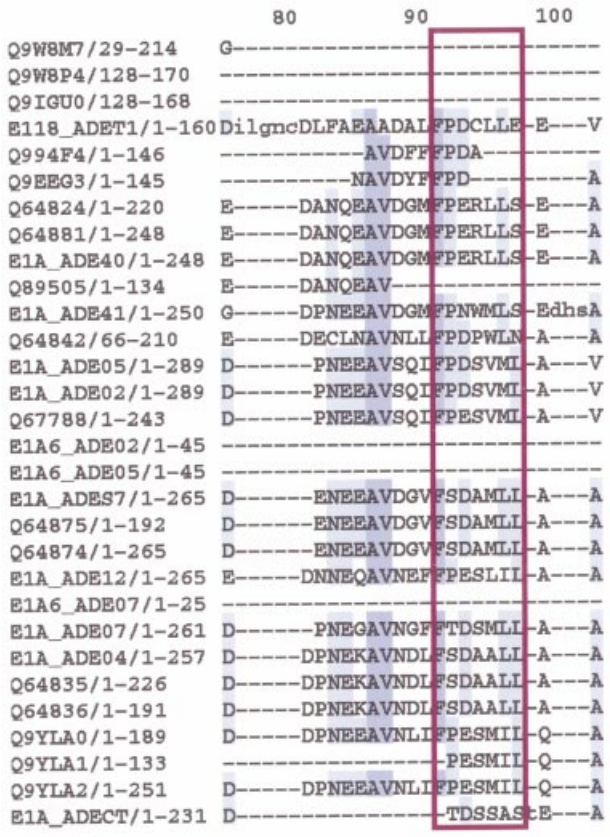

Figure 6. Location of the sequence pattern FX (D, E) XXXL (magenta box) in the multiple sequence alignment for the E1A family. The alignment was obtained from the Pfam database (39). The SwissProt (40) codes of the sequences are shown on the left side of the figure.

a well-defined sequence pattern, responsible for the binding to CBP, which is different from FX (D, E) XXXL. This would suggest the existence of a different binding mechanism for CBP/p300.

Our results point to the key role of the acetylase and deacetylase activities in viral growth promoted by T antigen. They show that a viral oncoprotein may manipulate gene expression by interfering with HAT chromatin-modifying enzymes. These observations highlight the relevance of acetylation/deacetylation pathways in the regulation of cell proliferation and viral infection.

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REFERENCES

1. Konberg,R.D. and Lorch,Y. (1999) Chromatin-modifying and remodelling complexes. *Curr. Opin. Genet. Dev.*, **9**, 148–151.
2. Kingston,R.E. and Narlikar,G.J. (1999) ATP-dependent remodelling and acetylation as regulators of chromatin fluidity. *Genes Dev.*, **13**, 2339–2352.
3. Wu,J. and Grunstein,M. (2000) 25 years after the nucleosome model: chromatin modifications. *Trends Biochem. Sci.*, **25**, 619–623.
4. Berger,S. (2002) Histone modifications in transcriptional regulation. *Curr. Opin. Genet. Dev.*, **12**, 142–148.
5. Hebbes,T.R., Thorne,A.W. and Crane-Robinson,C. (1988) A direct link between core histone acetylation and transcriptionally active chromatin. *EMBO J.*, **7**, 1395–1402.
6. Turner,B.M. and O'Neill,L.P. (1995) Histone acetylation in chromatin and chromosomes. *Semin. Cell Biol.*, **6**, 229–236.
7. Eckner,R., Ewen,M.E., Newsome,D., Gerdes,M., DeCaprio,J.A., Lawrence,J.B. and Livingston,D.M. (1994) Molecular cloning and functional analysis of the adenovirus E1A-associated 300-kD protein (p300) reveals a protein with properties of a transcriptional adapter. *Genes Dev.*, **8**, 869–884.
8. Bannister,A.J. and Kouzarides,T. (1996) The CBP co-activator is a histone acetyltransferase. *Nature*, **384**, 641–643.
9. Ogryzko,V.V., Schiltz,R.L., Russanova,V., Howars,B.H. and Nakatani,Y. (1996) The transcription coactivators p300 and CBP are histone acetyltransferase. *Cell*, **87**, 953–959.
10. Yang,X.-J., Ogryzko,V.V., Nishikawa,J., Howard,B. and Nakatani,Y. (1996) A p300/CBP-associated factor that competes with the adenoviral oncoprotein E1A. *Nature*, **382**, 319–324.
11. Chrivia,J.C., Kwok,R.P.S., Lamb,N., Hagiwara,M., Montminy,M.R. and Godman,R.H. (1993) Phosphorylated CREB binds specifically to the nuclear protein CBP. *Nature*, **365**, 855–859.
12. Dai,P., Akimaru,H., Tanaka,Y., Hou,D.X., Yasukawa,T., Kanei-Ishii,C., Takahashi,T. and Ishii,S. (1996) CBP as a transcriptional coactivator of c-Myb. *Genes Dev.*, **10**, 528–540.
13. Eckner,R., Yao,T.P., Oldread,E. and Livingston,D.M. (1996) Interaction and functional collaboration of p300/CBP and HLH proteins in muscle and B-cell differentiation. *Genes Dev.*, **10**, 2478–2490.
14. Trouche,D., Cook,A. and Kouzarides,T. (1996) The CBP co-activator stimulates E2F/DP1 activity. *Nucleic Acids Res.*, **24**, 4139–4145.
15. Gu,W. and Roeder,R.G. (1997) Activation of p53 sequence-specific DNA binding by acetylation of p53 C-terminal domain. *Cell*, **90**, 595–606.
16. Kamei,Y., Xu,L., Heinzel,T., Torchia,J., Kurokawa,R., Gloss,B., Lin,S.C., Heyman,R.A., Rose,D.W., Glass,C.K. and Rosenfeld,M.G. (1996) A CBP integrator complex mediates transcriptional activation and AP-1 inhibition by nuclear receptor. *Cell*, **85**, 403–414.
17. Kwok,R.P., Lundblad,J.R., Chrivia,J.C., Richards,J.P., Bachinger,H.P. Brennan,R.G., Roberts,S.G.E., Grenn,M.R. and Goodman,R.H. (1994) Nuclear protein CBP is a coactivator for the transcription factor CREB. *Nature*, **370**, 223–226.
18. Martínez-Balbás,M.A., Bannister,A.J., Martin,K., Haus-Seuffert,P., Meisterernst,M. and Kouzarides,T. (1998) The acetyltransferase activity of CBP stimulates transcription. *EMBO J.*, **17**, 2886–2893.
19. Chen,H., Lin,R.J., Schiltz,R.L., Chakravarti,D., Nash,A., Nagy,L., Privalsky,M.L., Nakatani,Y. and Evans,R.M. (1997) Nuclear receptor coactivator ACTR is a novel histone acetyltransferase and forms a multimeric activation complex with P/CAF and CBP/p300. *Cell*, **90**, 569–580.
20. Spencer,T.E., Jenster,G., Burcin,M.M., Allis,C.D., Zhou,J., Mizzen,C.A., McKenna,N.J., Onate,S.A., Tsai,S.Y., Tsai,M.J. and O'Malley,B.W. (1997) Steroid receptor coactivator-1 is a histone acetyltransferase. *Nature*, **389**, 194–198.

21. Moran,E. (1993) DNA tumor viruses and the cell cycle. *Curr. Opin. Genet. Dev.*, **3**, 63–70.
22. Ali,S.H. and DeCaprio,J.A. (2001) Cellular transformation by SV40 large T antigen: interaction with host proteins. *Semin. Cancer Biol.*, **11**, 15–22.
23. Levine,A.J. (1990) The p53 protein and its interaction with the oncogenic products of the small DNA tumor viruses. *Virology*, **77**, 419–426.
24. Sheppard,H.M., Corneillie,S.I., Espiritu,C., Gatti,A. and Liu,X. (1999) New insights into the mechanism of inhibition of p53 by simian virus 40 large T antigen. *Mol. Cell. Biol.*, **19**, 2746–2753.
25. DeCaprio,J.A., Ludlow,J.W., Figgel,J., Shew,J.Y., Huang,C.M., Lee,W.H., Marsilio,E., Paucha,E. and Livingston,D. (1988) SV40 large T antigen forms a specific complex with the product of the retinoblastoma susceptibility gene. *Cell*, **54**, 275–283.
26. Ludlow,J.W., Shon,J., Pipas,J.M., Livingston,D.M. and DeCaprio,J.A. (1990) The retinoblastoma susceptibility gene product undergoes cell cycle-dependent dephosphorylation and binding to and release from SV40 large T. *Cell*, **60**, 387–396.
27. Cobrinik,D., Dowdy,S.F., Hinds,P.W., Mittnacht,S. and Weinberg,R.A. (1992) The retinoblastoma protein and the regulation of cell cycling. *Trends Biochem. Sci.*, **71**, 310–315.
28. Dyson,N. and Harlow,E. (1992) Adenovirus E1a targets key regulators of cell proliferation. *Cancer Surv.*, **12**, 161–195.
29. Yaciuk,P., Carter,M.C., Pipas,J.M. and Moran,E. (1991) Simian virus 40 large antigen expresses a biological activity complementary to p300-associated transforming function of adenovirus E1A gene products. *Mol. Cell. Biol.*, **4**, 2116–2124.
30. Eckner,R., Ludlow,J.W., Lill,N.L., Oldread,E., Arany,Z., Modjtahedi,N., DeCaprio,J.A., Livingston,D.M. and Morgan,J.A. (1996) Association of p300 and CBP with simian virus 40 large T antigen. *Mol. Cell. Biol.*, **16**, 3454–3464.
31. Avantaggiati,M.L., Carbone,M., Graessmann,A., Nakatani,Y., Howard,B. and Levine,S.A. (1996) The SV40 large T antigen and adenovirus E1a oncoproteins interact with distinct isoforms of the transcriptional co-activator, p300. *EMBO J.*, **15**, 2236–2248.
32. Lill,N.L., Tevethia,M.J., Eckner,R., Livingston,D.M. and Modjtahedi,N. (1997) p300 family members associate with the carboxyl terminus of simian virus 40 large tumor antigen. *J. Virol.*, **71**, 129–137.
33. Arany,Z., Newsome,D., Oldread,E., Livingston,D. and Eckner,R. (1995) A family of transcriptional adapter proteins targeted by the E1a oncoprotein. *Nature*, **374**, 81–84.
34. Hamamori,Y., Sartorelli,V., Ogryzko,V., Puri,P.L., Wu,H.Y., Wang,J.Y., Nakatani,Y. and Kedes,L. (1999) Regulation of histone acetyltransferases p300 and PCAF by the bHLH protein twist and adenoviral oncoprotein E1A. *Cell*, **59**, 405–413.
35. Chakravarti,D., Ogryzko,V., Kao,H.-Y., Nash,A., Chen,H., Nakatani,Y. and Evans,R.M. (1999) A viral mechanism for the inhibition of p300 and P/CAF acetyltransferase activity. *Cell*, **96**, 393–403.
36. Ait-Si-Ali,S., Ramirez,S., Barre,F.-X., Dkhissi,F., Magnaghi-Jaulin,L., Girault,J.A., Robin,P., Knibiehler,M., Pritchard,L.L., Ducommun,B., Trouche,D. and Harel-Bellan,A. (1998) Histone acetyltransferase activity of CBP is controlled by cycle-dependent kinases and oncoprotein E1A. *Nature*, **396**, 184–186.
37. Lavender,P., Vandel,L., Bannister,A.J. and Kouzarides,T. (1997) The HMG-box transcription factor HBP1 is targeted by the pocket proteins and E1A. *Oncogene*, **14**, 2721–2728.
38. Su,R.T. and DePamphilis,M.L. (1978) Simian virus 40 DNA replication in isolated replicating viral chromosomes. *J. Virol.*, **28**, 53–65.
39. Bateman,A., Birney,E., Cerruti,L., Durbin,R., Etwiller,L., Eddy,S.R., Griffiths-Jones,S., Howe,K.L., Marshall,M. and Sonnhammer,E.L. (2002) The Pfam protein families database. *Nucleic Acids Res.*, **30**, 276–280.
40. Bairoch,A. and Apweiler,R. (2000) The SWISS-PROT protein sequence database and its supplement TrEMBL in 2000. *Nucleic Acids Res.*, **28**, 45–48.
41. Hebbes,T.R., Thorne,A.W. and Crane-Robinson,C. (1988) A direct link between core histone acetylation and transcriptionally active chromatin. *EMBO J.*, **7**, 1395–1402.
42. Reid,L.J., Bannister,A.J., Zagerman,P., Martínez-Balbás,M.A. and Kouzarides,T. (1998) E1A directly binds and regulates the P/CAF acetyltransferase. *EMBO J.*, **17**, 4469–4477.
43. Alberts,A.S., Geneste,O. and Treisman,R. (1998) Activation of SRF-regulated chromosomal templates by Rho-family GTPases requires a signal that also induces H4 hyperacetylation. *Cell*, **92**, 475–487.
44. Vogelauer,M., Wu,J., Suka,N. and Grunstrein,M. (2000) Global histone acetylation and deacetylation in yeast. *Nature*, **408**, 495–498.
45. Howe,L., Auston D., Grant,P., John,S., Cook,R.G., Workman,J.L. and Pillus,L. (2001) Histone H3 specific acetyltransferase are essential for cell cycle progression. *Genes Dev.*, **15**, 3144–3154.
46. Kristjuban,A., Walker,J., Suka,N., Grunstein,M., Roberts,D., Cairns,B.R. and Svejstrup,J.Q. (2002) Transcriptional inhibition of genes with severe histone H3 hypoacetylation in the coding region. *Mol. Cell*, **10**, 925–933.
47. O'Connor,M., Zimmermann,H., Nielsen,S., Bernard,H. and Kouzarides,T. (1999) Characterization of an E1A–CBP interaction defines a novel transcription adapter motif (TRAM) in CBP/p300. *J. Virol.*, **73**, 3574–3581.
48. Altschul,S.F., Madden,T.L., Schaffer,A.A., Zhang,J., Zhang,Z., Miller,W. and Lipman,D.J. (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search program. *Nucleic Acids Res.*, **25**, 3389–3402.