E1A12S-mediated Activation of the Adenovirus Type 12 E2 Promoter Depends on the Histone Acetyltransferase Activity of p300/CBP*

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Activation of the transcription unit early region 2 (E2) promoter of the oncogenic adenovirus serotype 12 (Ad12), which regulates the expression of proteins essential for viral replication, requires the assembly of a ternary complex consisting of cAMP response element-binding protein (CREB)-1/activating transcription factor (ATF)-1, the Ad12 12S oncogene product of early region 1A (E1A12S), and the co-activator p300/CBP on the E2 Ad12 cAMP response element (E2-CRE). Here we show that the active E2A12 promoter is associated with acetylated histone H4 whereas an E2-CRE point-mutated promoter which is transcriptionally inactive due to its inability to assemble this ternary complex is not bound by acetylated histone H4. The histone deacetylase 1 as well as Roscovitine, which blocks the activation of the histone acetyltransferase (HAT) activity of CBP by cyclin E-Cdk2, prevents E2A12 promoter activation through E1A12S. p300/CBP counteracts the repressive function of histone deacetylase 1 in a HAT domain-dependent manner whereas the p300/CBP-associated factor PCAF failed to rescue E2A12 promoter activity. E1A12S-bound p300/CBP displays strong HAT activity. Most interestingly, E1A12S-mediated activation of the E2A12 promoter correlates well with the ability of the viral protein to associate with the HAT activity of p300/CBP in vivo. Taken together these data indicate that the recruitment of the HAT activity of p300/CBP by E1A12S plays an important role in E2A12 promoter activation.

Nuclear integrators of diverse signaling pathways like p300 and CREB-binding protein (CBP) play crucial roles in the coordinated regulation of gene expression (1). Although it turns out recently that p300 and CBP have partially different biological properties (2–4) they are referred here to as p300/CBP unless otherwise specified due to their indistinguishable function on the activation of CREB-dependent gene expression (5). p300/CBP binds to and potentiates the function of a large number of transcription factors, including CREB (5) and Ad E1A (6). In addition, it interacts with other co-factors like PCAF (7), SRC-1 (8), or pCIP (9) for promoter activation. Moreover, at least CBP was shown to associate directly with the RNA polymerase II holoenzyme via RNA helicase A (10). Latter interaction is thought to induce local changes in chromatin structure that promote access of the transcriptional machinery on responsive promoters.

The finding that p300/CBP carries intrinsic HAT activity (11, 12) was a major step forward in unraveling one mechanism how co-factors could function in transcriptional regulation. As nucleosomes are potent repressors of transcription in vitro and in vivo it is assumed that acetylation of histones disrupts the nucleosomal structure thereby facilitating access of DNA-binding factors and the transcriptional apparatus (13). p300/CBP has also been shown to acetylate non-histone proteins, like general as well as sequence-specific transcription factors (14–17). These acetylations increase DNA binding activity (14, 16), decrease DNA binding activity (17), or prevent protein/protein interactions (18).

The Ad E1A oncoprotein is a potent transcriptional regulator (19) which interferes with a variety of cellular processes such as modulation of gene expression, inhibition of cellular differentiation, promotion of cell-cycle progression, and transformation (20, 21). Subsequent studies have clearly demonstrated a correlation between the modulation of these processes and the functional interaction of E1A with p300/CBP (Ref. 6, and references therein). Molecular analyses identified three binding domains within p300/CBP for E1A (22, 23): (i) aa 1–450, (ii) aa 1459–1891 (spanning the C/H3 domain), and (iii) aa 2058–2163. Interestingly, the C/H3-binding site is located immediately adjacent to the HAT domain of p300/CBP suggesting that E1A might affect cellular processes by interfering with this enzymatic activity. However, the modulation of the p300/CBP HAT activity by E1A remains unclear. On the one hand, several reports suggest that E1A inhibits the HAT activity of p300 (24, 25), whereas on the other hand no modulation (11) or even a stimulation of CBP’s HAT activity through the adenoviral protein was reported (26).

The aim of this study was to analyze the functional relationship between the Ad E1A12S protein and the HAT activity of p300/CBP in the process of the E2A12S promoter activation. The Ad E2 gene encodes for proteins essential for viral replication and its expression is therefore of fundamental importance for the viral life cycle (27). We have recently shown that the E1A12S protein of Ad12 activates the E2A12S promoter through the E2-CRE (28). Furthermore, our results indicate that transcriptional activation of this viral promoter requires the assembly of a ternary complex consisting of CREB-1/ATF-1, E1A12S, and CBP on the E2-CRE. However, the viral genome is rapidly...
chromatinized after infection (Refs. 29 and 30, and references therein). Therefore the question arises whether E1A12S-mediated activation of the E2Ad12 promoter requires the HAT activity of p300/CBP. Here we show that HDAC-1 and Roscovitine, which blocks CBP HAT activation (26), suppress the E1A12S-mediated activation of the E2Ad12 promoter and that p300/CBP counteracts the HDAC-1-mediated repression in a HAT domain-dependent manner. In contrast, the histone acetyltransferase PCAF failed to counteract the HDAC-1-mediated repression indicating the necessity of a specific HAT activity for promoter activation. The active E2Ad12 promoter is associated with acetylated histone H4 whereas an E2Ad12 mutant promoter rendered inactive through a mutation in the E2-CRE is not associated with acetylated H4. Most interestingly, activation of the E2Ad12 promoter through E1A12S correlates well with an association of the adenosiviral protein with the HAT activity of p300/CBP. Taken together our data suggest that recruitment of the HAT activity of p300/CBP by E1A12S and acetylation of promoter bound histones are important steps in the activation process of the E2Ad12 promoter.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—The expression vectors pRc/RSV-235R and pRc/RSV-Gal4-E1A12S encoding the E1A12S protein of Ad12 E1A or a Gal4-E1A12S fusion protein, respectively, were described elsewhere (31, 32). Expression vectors encoding the E1A12S mutants as Myc/His-tagged fusions were constructed by cloning their respective cDNAs amplified by PCR into the EcoRI/BamHI sites of pDNA3.1-A-Myc/His (Invitrogen). The bacterial and eukaryotic E1A expression vectors as well as the reporter constructs E2Ad12-CAT and E2Ad12pmCRE-CAT have been described (23, 28).

**Cell Culture**—KB (human oral epidermoid carcinoma cells), HeLa (human cervix carcinoma cells), and COS cells (SV40-transformed African green monkey kidney cells) were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum, penicillin, and streptomycin at 37 °C and 5% CO2.

**Transient Expression Assays**—For transient expression assays 2.5 × 105 KB or HeLa cells were co-transfected with 1 μg of the respective E2Ad12 reporter construct and different amounts of expression vectors as indicated in the Figure legends using Polyfectine according to the manufacturer’s instructions (Biontex, Martinsried, Germany). Transfections were stopped after 6 h and cells were harvested 24 h later to determine CAT activity (33). In some experiments Roscovitine was added to the medium 1 h before harvesting.

**Combined GST Pull-down and HAT Assays**—GST fusion proteins were expressed in Escherichia coli BL21 bacteria and purified as described previously (26). E1A antiserum as described previously (28). Protein A/G-Sepharose (Amersham Pharmacia Biotech) for 2 h at 4 °C, and sonicated 5 times for 10 s and bound histones are important steps in the activation process of the E1A12S protein.

**RESULTS**

**HDAC-1 as Well as the CBP Inhibitor Roscovitine Inhibit the E1A12S-mediated Activation of the E2Ad12 Promoter**—To analyze whether the activity of the E2Ad12 promoter is modulated in an acetylation/deacetylation-dependent manner we performed transient expression assays. The reporter construct E2Ad12-CAT which carries the minimal promoter inducible by E1A12S (Fig. 1A; Ref. 28), was co-transfected with expression vectors coding for E1A12S and/or HDAC-1 in KB cells. CAT activity was determined 24 h later. As shown in Fig. 1A, HDAC-1 strongly represses the E1A12S-mediated CAT gene expression indicating that histone acetylation plays an important role in the activation of the E2Ad12 Promoter. To confirm this assumption we made use of the drug Roscovitine. It has been shown that Roscovitine blocks proliferation signals which mediate CBP HAT activation (26). E2Ad12-CAT was transfected in KB cells and Roscovitine was added at the indicated concentrations (Fig. 1B) 14 h before harvesting the cells. The E1A12S mediated activation of the E2Ad12 promoter in the absence of Roscovitine was set as 100% in these experiments (Fig. 1B). Roscovitine inhibits the E1A12S-mediated activation of the E2Ad12 promoter in a dose-dependent manner and nearly completely blocks CAT-gene expression at a concentration of 25 μM (Fig. 1B). To exclude that Roscovitine inhibits indirectly the activation of the E2Ad12 promoter by interfering with the expression of E1A12S we made use of the Gal4 expression system. The reporter construct G5-E1BTATA-CAT which contains five binding sites for the yeast transcription factor Gal4 was co-transfected with pRc/RSV-Gal4-E1A12S at KB cells in the presence of Roscovitine (50 μM). Roscovitine (50 μM) blocked Gal4-CAT promoter activity of the Gal4 DNA-binding domain fused to the E1A12S protein. Of note, its expression was controlled by the same SV40 promoter as E1A12S used in the experiments summarized in Fig. 1A. Gal4-E1A12S strongly activates CAT gene expression from G5-E1BTATA-CAT (Fig. 1C; Ref. 32). Roscovitine does not repress the Gal4-E1A12S-dependent activation of the G5-E1BTATA promoter (Fig. 1C) indicating that this drug inhibits the E2 promoter directly and not via an indirect mechanism. Furthermore, Western blot analyses dem-
shown a comparable E1A12S protein concentration in transiently transfected KB cells in the presence or absence of Roscovitine (data not shown). Taken together these data suggest that the activity of the E2Ad12 promoter is modulated in an acetylation/deacetylation-dependent mechanism.

p300/CBP Counteracts the HDAC-1-mediated Repression of the E2Ad12 Promoter—As E1A12S recruits p300/CBP to the E2Ad12 promoter (28) we asked next if this co-factor might be able to counteract the inhibition of the E2Ad12 Promoter mediated by the histone deacetylase HDAC-1. We therefore performed transient expression assays co-transfecting the E2Ad12-CAT reporter construct with expression vectors for HDAC-1, p300/CBP, and/or E1A12S. For this experiment the concentration of each expression vector was titrated to be able to measure the effect of E1A12S as well as p300/CBP. As already shown in Fig. 1A, HDAC-1 represses the E1A12S-mediated activation of the E2Ad12 Promoter (Fig. 2, A and B). p300 as well as CBP restores E1A12S-dependent transactivation of the E2Ad12 promoter in the presence of HDAC-1 (Fig. 2, A and B). Most importantly, a CBP mutant which is completely defective in HAT activity due to a deletion of an 1431–1569 in the HAT domain (37) failed to counteract the HDAC-1-mediated repression (Fig. 2B). This finding demonstrates the necessity of the HAT activity of p300/CBP for promoter activation in the presence of HDAC-1 and excludes the possibility that the co-factor circumvents the HDAC-1-mediated repression by a HAT-domain independent mechanism. In the presence of the mutant Δ1–79/E1A12S, which lacks an 1–79 essential for the activation of the E2Ad12 promoter (Fig. 2A, Ref. 28) as well as for the binding to both co-factors (Fig. 6B; Ref. 28), p300/CBP failed to rescue the HDAC-1-mediated repression (Fig. 2A) suggesting that the recruitment of their HAT activity through E1A12S to the E2Ad12 promoter is necessary to counteract the HDAC-1 effect. Interestingly, p300/CBP does not activate the E2Ad12 promoter in the absence of HDAC-1 indicating that the endogenous co-activator concentration is not limiting with respect to promoter activation in the absence of overexpressed HDAC-1 under the conditions used (Fig. 2A). These data are consistent with observations of Chen and co-workers (38) who analyzed the activation of hormone-dependent gene expression through p300. Finally, the HAT P/CAF, which binds to E1A12S (39) as well as to p300/CBP (7) failed to counteract the HDAC-1-mediated E2Ad12 promoter repression (Fig. 2A). This result suggests that the activation of the E2Ad12 promoter depends on a specific HAT activity, namely on that of p300/CBP. Taken together these results support our hypothesis that the HAT activity of p300/CBP is necessary for the activation of the E2Ad12 promoter.

**E2Ad12 Promoter Activity Is Linked to the Acetylation Status of Histone H4**—We have shown previously that a point mutation in the E2-CRE prevented promoter activation (28) due to its inability to assemble the ternary complex consisting of vectors was set as 1. The E2Ad12 promoter is schematically shown at the top of the figure. B, KB cells were transiently transfected with 1 μg of the E2Ad12-CAT reporter construct and 1.5 μg of pCMV-HA-HDAC-1 and 0.3 μg of pRc/RSV-E1A12S as indicated. Empty expression vectors were added to keep the amount of transfected DNA constant. The results are the average of three independent experiments performed in duplicate with standard deviations indicated. The promoter activity of E2Ad12-CAT in the presence of empty

![Fig. 1. HDAC-1 as well as the CBP inhibitor Roscovitine repress E1A12S-mediated activation of the E2Ad12 Promoter.](http://www.jbc.org/content/275/38/40556/F1)

**Fig. 1.** HDAC-1 as well as the CBP inhibitor Roscovitine repress E1A12S-mediated activation of the E2Ad12 Promoter. A, KB cells were co-transfected with 1 μg of the E2Ad12-CAT reporter construct, 1.5 μg of pCMV-HA-HDAC-1 and 0.3 μg of pRc/RSV-E1A12S as indicated. Empty expression vectors were added to keep the amount of transfected DNA constant. The results are the average of three independent experiments performed in duplicate with standard deviations indicated. The promoter activity of E2Ad12-CAT in the presence of empty vector was set as 1. The promoter activity of G5-E1BTATA-CAT in the presence of pRc/RSV-E1A12S was determined. The results are the average of three independent experiments performed in duplicate with standard deviations indicated. The promoter activity of E2Ad12-CAT in the presence of pRc/RSV-E1A12S with/out Roscovitine was set as 100%. C, KB cells were transiently transfected with 0.2 μg of G5-E1BTATA-CAT and 1 μg of Gal4-E1A12S expression vector or empty expression vector as indicated at the bottom of the figure. Roscovitine was added at a concentration of 25 μM 10 h after transfection (open bars). The promoter activity of G5-E1BTATA-CAT in the presence of empty vector was set as 1. The reporter construct is schematically drawn at the top of the figure. 5XGal4 represents five binding sites for the yeast Gal4 transcription factor.
CREB-1/ATF-1, EIA12S, and p300/CBP. As p300/CBP counteracts the repression mediated by HDAC-1 we wanted to know if loss of promoter activity through the point mutation in the E2-CRE correlates with a loss of histone acetylation. To address this question we made use of chromatin immunoprecipitation analyses. The E2a12S reporter construct was transfected in KB cells followed by immunoprecipitations with an anti-acetylated histone H4 antibody (lanes 4 and 5) or control antibody (normal rabbit IgG, lane 3) and analyzed by PCR using primers specific for both promoter fragments. Aliquots of the soluble chromatin were analyzed before immunoprecipitation. The positions of the E2a12S promoter fragments are indicated on the right, the molecular weight marker on the left. Lane 6 represents the PCR control reaction without template.

**FIG. 3.** The active E2a12S promoter is associated with acetylated histone H4 in vivo. Soluble chromatin from KB cells transfected with either E2a12S-CAT (lanes 1, 3, and 4) or E2a12SpmCRE-CAT (lanes 2 and 5) was immunoprecipitated with an anti-acetyl H4 antibody (lanes 4 and 5) or control antibody (normal rabbit IgG, lane 3) and analyzed by PCR using primers specific for both promoter fragments. Aliquots of the soluble chromatin were analyzed before immunoprecipitation (Total; lanes 1 and 2). The positions of the E2a12S promoter fragments are indicated on the right, the molecular weight marker on the left. Lane 6 represents the PCR control reaction without template.

CREB-1/ATF-1, EIA12S, and p300/CBP. As p300/CBP counteracts the repression mediated by HDAC-1 we wanted to know if loss of promoter activity through the point mutation in the E2-CRE correlates with a loss of histone acetylation. To address this question we made use of chromatin immunoprecipitation analyses. The E2a12S reporter construct was transfected in KB cells followed by immunoprecipitations with an anti-acetylated histone H4 antibody. Precipitated E2a12S promoter DNA was detected by PCR using primers recognizing specifically the E2a12S promoter through EIA12S.

p300/CBP Displays HAT Activity if Bound to EIA12S—Our data indicate that the HAT activity of p300/CBP plays an important role in the EIA12S-mediated activation of the E2a12S promoter. However, controversial data are published concerning the modification of the HAT activity of p300/CBP by EIA12S (11, 24–26). Therefore it was important for the understanding of the activation mechanism of the E2a12S promoter to clarify if p300/CBP bound to EIA12S of oncogenic Ad12 displays HAT activity at all. To address this question an immobilized GST-E1A12S fusion protein was incubated with cellular extract prepared from KB cells and bound protein was subjected to liquid HAT assays using either a H4-peptide or a histone/protein mixture consisting of H2A, H2B, H3, and H4 as substrates. These experiments revealed that the GST-E1A12S fusion protein associates with a cellular HAT activity (Fig. 4A), which acetylates at least histones H3 and H4 (Fig. 4B, lane 2). In contrast the GST leader sequence did not bind a HAT (Fig. 4, A and B, lane 1) confirming that the measured HAT activity is due a cellular factor(s) binding to the adenoviral part of the fusion protein. To identify these HATs, aliquots of the GST pull-down assays were analyzed by Western blots. EIA12S pulled down p300 and CBP (Fig. 4C, lane 3) whereas an EIA12S mutant that lacks the p300/CBP interaction domains (aa 1–79; Ref. 28) and which therefore failed to bind both cellular co-activators (Fig. 4C, lane 4, Ref. 28) did not associate with a HAT activity (Fig. 4, A and B, lane 3). Moreover, the p300/CBP-associated factor PCAF, which has also been shown to bind to the EIA protein and which carries intrinsic HAT activity, too (7, 39), was not found to be complexed with EIA12S under these conditions (data not shown). These data show that EIA12S recruits a HAT activity from cellular extracts which is most probably due to associated p300/CBP.

To further analyze whether a direct physical interaction between EIA12S and p300/CBP has any impact on the enzymatic activity of the cellular co-factor, HAT assays were performed in which recombinant and purified GST-CBP-1–1890 (20 pmol) was incubated with rising concentrations of a bacterially expressed and purified Hist6-E1A12S fusion protein (180 pmol to 2.3 nmol) in the presence of a biotinylated synthetic H4 peptide (0.67 mmol) as substrate. CBP-1–1890 carries the N terminus of CBP including the CBP1–1890 carries the N terminus of CBP including the

**FIG. 2.** p300 counteracts the HDAC-1-mediated repression of the E2a12S promoter. A, KB cells were co-transfected with 1 μg of the E2a12S-CAT reporter construct, 1 μg of pCMV-RA-HDAC-1, 0.1 μg of CMVβ-p300, 0.5 μg pCX-PCAF, 0.3 μg of pRe/RSV-EIA12S, or 0.3 μg of pRe/RSV-A1–79/EIA12S or in combination as indicated. Empty expression vectors were added to keep the amount of transfected DNA constant. The results are the average of three independent experiments performed in duplicate with standard deviations indicated. The promoter activity of E2a12S-CAT in the presence of empty vectors was set as 1. B, HeLa cells were co-transfected with 1 μg of the E2a12S-CAT reporter construct, 1 μg of pCMV-RA-HDAC-1, 0.2 μg of pRe/RSV-CBP, 0.2 μg of pCMV-2NT3-CBPΔHAT, 0.3 μg of pRe/RSV-EIA12S, or in combination as indicated. Empty expression vectors were added to keep the amount of transfected DNA constant. The results are the average of three independent experiments performed in duplicate with standard deviations indicated. The promoter activity of E2a12S-CAT in the presence of empty vectors was set as 1.
Thereafter HAT activity decreases and at a 115-fold molar excess, His$_6$-E1A$_{12S}$ reduced the HAT activity of CBP$_{1-1890}$ below the activity observed in the absence of the viral protein (repression factor: 2-fold; Fig. 5A; see also “Discussion”). Neither the GST leader sequence nor His$_6$-E1A$_{12S}$ displayed detectable HAT activity (data not shown) demonstrating that the measured enzymatic activity is due to the intrinsic HAT activity of CBP$_{1-1890}$. Interestingly, the HAT activity of a CBP mutant lacking the C/H3 domain (GST-CBP$_{1-1690}$), which is comparable to the HAT activity of CBP$_{1-1890}$ in the absence of the viral protein (data not shown), was not regulated by His$_6$-E1A$_{12S}$ (Fig. 5B). From these results we conclude that E1A$_{12S}$ might modulate the HAT activity of CBP in a dose-dependent manner in vitro and that a direct interaction between CBP and E1A$_{12S}$ via the C/H3 domain of the co-activator is necessary for modulation of the enzymatic activity.

E1A$_{12S}$-mediated Activation of the E2$_{Ad12}$ Promoter Correlates with the Ability of the Viral Protein to Associate with the HAT Activity of p300/CBP in Vivo—

We next asked if E1A$_{12S}$ of Ad12 associates with a HAT activity in vivo. COS7 cells were transiently transfected with the Myc epitope-tagged E1A$_{12S}$ wild type or mutants. Immune complexes obtained by usage of an anti-Myc mouse monoclonal antibody were analyzed for their associated HAT activity. In parallel experiments the ability of p300/CBP to associate with the E1A$_{12S}$ mutants in vivo was analyzed by immune coprecipitations. E1A$_{12S}$ and the point mutants R2G/E1A$_{12S}$ (substitution of arginine at position 2 by glycine) and D24A/E1A$_{12S}$ associate with strong HAT activity (Fig. 6A) and precipitate with p300/CBP (Fig. 6B; Ref. 28). In contrast, the deletion mutants D$_{N}$/E1A$_{12S}$ (lacking aa 1–29) and D$_{1–79}$/E1A$_{12S}$ (lacking aa 1–29 and the conserved region 1) have lost their ability to bind to p300/CBP (Fig. 6B; Ref. 28) and to associate with a strong HAT activity (Fig. 6A). The mutants DCR1/E1A$_{12S}$ (lacking the conserved region 1), I$_{18}$/E1A$_{12S}$, and L$_{19}$/E1A$_{12S}$ have a greatly reduced capacity to associate with p300/CBP (Fig. 6B; Ref. 28) and to associate with a HAT (Fig. 6A). Interestingly, the point mutant D16A/E1A$_{12S}$ does not bind p300/CBP (Fig. 6B; Ref. 28) but precipitates with a significant HAT activity (Fig. 6A). However, this HAT activity might be due to a different enzyme like PCAF.
E1A12S wild type protein as well as mutants R2G/E1A12S and with a HAT activity (Fig. 6A) and do not activate the E2Ad12-promoter (23, 28) do not or only slightly associate with p300/CBP (Fig. 6B) (7, 39) which precipitate with the E1A12S mutant and Table I) (23, 28), whereas the E1A12S-protein Activation of the E2Ad12-promoter, which might interact with the E1A12S point mutant (see “Discussion”). All E1A mutants were expressed in a comparable amount to the wild type protein as determined by Western blotting (data not shown), excluding that the loss or reduction of their association with a HAT activity is due to a reduced expression. The association of the E1A12S wild type and mutant proteins with a HAT activity and p300/CBP correlates well with the activation of the E2Ad12 promoter (summarized in Table I). The E1A12S wild type protein as well as mutants R2G/E1A12S and D24A/E1A12S bind efficiently to p300/CBP (Fig. 6B) (23, 28), activate strongly the E2Ad12 promoter (Table I) (28) and are associated with a strong HAT activity (Fig. 6A), whereas the mutants ΔN/E1A12S, and Δ1–79/E1A12S, which do not bind to p300/CBP (Fig. 6B) (23, 28) do not or only slightly associate with a HAT activity (Fig. 6A) and do not activate the E2Ad12 promoter (Table I) (28). Interestingly, the mutants ΔCR1/E1A12S, Δ16A/E1A12S, I18P/E1A12S, and L19S/E1A12S, which show either a marginal or no interaction with p300/CBP or activation of the E2Ad12 promoter (Fig. 6B and Table I) (23, 28), associate with a residual HAT activity (Fig. 6A). However, this remaining HAT activity might be due to other cellular HATs like PCAF (7, 39) which precipitate with the E1A12S mutant proteins instead of p300/CBP. Taken together these data demonstrate a good correlation between the ability of the E1A12S protein to associate with a HAT activity, to bind to p300/CBP, and to activate the E2Ad12 promoter. Moreover these data support our hypothesis that recruitment of the p300/CBP HAT activity through the adenoviral protein is necessary for E2Ad12 promoter activation.

### DISCUSSION

Activation of viral gene expression is of crucial importance for propagation of Ads. However, the Ad genome is rapidly chromatinized after infection (29) and nucleosomes repress gene expression (13). To solve this paradoxical situation the chromatin-like structure of the Ad genome has to be remodeled in order to increase access of general transcription factors which in turn results in a strong activation of target gene expression. An attractive model system to proof this hypothesis is the E2 Ad12 promoter which drives the expression of genes necessary for Ad replication (27).

A prerequisite for the activation of the E2Ad12 promoter is the assembly of a ternary complex consisting of CREB-1/ATF-1, E1A12S, and p300/CBP on the E2-CRE (28). The role of E1A12S in this complex is most probably multifunctional. The viral protein might interact directly with factors of the general transcription machinery like TATA box-binding protein and/or the RAP30 subunit of TFII F. Both factors are important for promoter activation (40) and both bind to the N-terminal transactivation domain of E1A12S (41). In addition, E1A12S might recruit the HAT activity of p300/CBP. Our data presented here support the latter hypothesis. First, activation of the E2Ad12 promoter correlates well with the ability of the E1A12S protein to associate with the HAT activity of p300/CBP. The point mutant D16A/E1A12S seems to be an exception from this general scheme as it associates with a HAT activity but activates only slightly the E2Ad12 promoter. However, D16A/E1A12S does not interact with p300/CBP in vivo. We therefore conclude that the associated HAT activity is most probably due to a different HAT like PCAF which is able to bind to the E1A12S mutants but is unable to support significantly the E2Ad12 promoter activation (see below). Second, our data show a correlation between E2Ad12 promoter activity and the acetylation status of histones bound to the E2Ad12 promoter. The active E2Ad12 promoter is associated with acetylated histone H4 whereas a point mutated E2Ad12 promoter, which is transcriptionally inactive due to its inability to recruit the ternary complex, is not bound by acetylated histones. Furthermore, HDAC-1 and Roscovitine repress E1A12S induced promoter activation which can be restored by either the HDAC-1 inhibitor trichostatin A (data not available).
observed that a large molar excess of E1A12S (115-fold) inhibits binding to its minimal HAT domain (24). Interestingly, we hematolytic activity of p300/CBP in the process of E2Ad12 promoter mutant lacking the classical binding motif for p300/CBP (aa G1/S boundary of the cell cycle by phosphorylation of specific Cyclin E-Cdk2 activates the enzymatic activity of CBP at the activation. However, the activation mechanism is not yet clear.

We observed that an interaction between E1A12S and the C/H3 domain might also induce a conformational change thus leading to an increase of the enzymatic activity.

CBP. This idea is supported by the finding of Chakravarti et al. (24) who have shown that E1A is efficiently acetylated by p300/CBP in vitro.

Several models are proposed how HATs might affect transcriptional activity (13). In one model, HATs are generally targeted to promoters because of their ability to associate with a general component of the Pol II transcription machinery. Our results represent an example for a model in which HATs selectively affect gene expression through their recruitment to particular promoters. According to this model, p300/CBP is tethered to the E2-CRE through the concerted action of E1A12S and CREB-1/ATF-1. In addition to recruiting the co-activator, E1A12S might also increase the enzymatic activity of p300/CBP. Together with several putative direct interactions of E1A12S and/or CREB-1/ATF-1 with general transcription factors, this process might be responsible for the activation of the E2Ad12 promoter.

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