cAMP Response Element-binding Protein-binding Protein Binds to Human Papillomavirus E2 Protein and Activates E2-dependent Transcription*

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cAMP response element-binding protein (CREB)1-binding protein (CBP) is a eucaryotic transcriptional co-activator that contains multiple protein-protein interaction domains for association with various transcription factors, components of the basal transcriptional apparatus, and other co-activator proteins. Here, we report that CBP is also a co-activator of the human papillomavirus (HPV) E2 protein, which is a sequence-specific transcription/repllication factor. We provide biochemical, genetic, and functional evidence that CBP binds directly to HPV E2 in vivo and in vitro and activates E2-dependent transcription. Mutations in an amphipathic helix within HPV-18 E2 abolish its transcriptional activation properties and its ability to bind to CBP. Furthermore, the binding of CBP to E2 was shown to be necessary for E2-dependent transcription. Interestingly, the histone acetyltransferase activity of CBP plays a role in CBP activation of E2-dependent transcription.

DNA tumor viruses encode proteins that inappropriately activate host cell DNA synthesis by interacting with and inhibiting the activity of cellular proteins that normally function to repress cell proliferation. Examples of such viral proteins include the adenovirus E1A protein and the SV40 large T antigen. Recently, it was reported that E1A and SV40 large T antigen can bind to CBP and affect its functions (11, 12), and a role for CBP in cellular growth control has been proposed on the basis of such experiments (13). E1A and large T antigen mutant proteins that cannot bind to CBP are defective in the induction of cellular DNA synthesis (12, 14, 15). CBP has been shown to be required for the activation of muscle-specific genes and for cell cycle arrest during differentiation of muscle cells. Thus, CBP can function as a negative regulator of cell growth, and E1A may carry out its mitogenic and oncogenic functions by binding to CBP and inhibiting cellular growth-restraining pathways (16–18). A second cellular protein that interacts with E1A is p300. p300 was first discovered in anti-E1A immunoprecipitates (11). p300 displays a striking sequence similarity with CBP and can substitute for CBP in potentiating CREB-activated gene expression (19).

The papillomavirus (PV) E2 open reading frame encodes several proteins that bind to the consensus E2-binding sequence (E2BS) ACCN₆GGT and regulate viral transcription and DNA replication (20, 21). Full-length E2 protein can support viral transcription and DNA replication, whereas alternatively spliced forms of the E2 protein that lack the amino-terminal domain act as repressors of transcription (22, 23). Analysis of the amino acid sequences of various E2 proteins shows that the NH₂-terminal and COOH-terminal regions are relatively well conserved (20, 21). A transactivation domain is encoded by the conserved NH₂-terminal region, and a DNA binding domain is encoded by the conserved COOH-terminal region. The activation domain of bovine papillomavirus type 1 E2 contains two regions within the first 85 amino acids that are predicted to form acidic amphipathic helices (24). It has been proposed that the E2 amphipathic helices might be responsible for interaction with host transcriptional modulators (24, 25).

The E2 gene product appears to play an inhibitory role in human papillomavirus (HPV)-induced carcinogenesis. The great majority of human cervical cancers contain integrated HPV DNA and express the HPV E6 and E7 oncoproteins, which exert their proliferative effects by binding to and inactivating the tumor suppressor proteins p53 and pRb, respectively (26, 27). In contrast, the E2 gene is usually disrupted in cervical cancers, suggesting that loss of the E2 protein is an important step in the development of cervical cancers (28, 29). It has been shown that E2 increases the concentration of p53 protein and induces a G₁ arrest in HeLa cells, a cervical carcinoma cell line (30).

Because there is functional cross-talk between CBP and a number of cellular transcription factors, we sought to deter-
mine whether HPV E2 and CBP physically interact. Herein, we show that HPV-18 E2 and CBP directly interact to form a specific protein complex and that E2-mediated transactivation is CBP-dependent. Mutations in the amphipathic helix of HPV-18 E2 abolished the transactivation properties of E2 and revealed that binding of CBP to E2 is necessary for E2-dependent transactivation. Finally, we show that the IAT activity of CBP is involved in E2-dependent transactivation.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—p6xE2BstkCAT and pCMV4/16E2 were gifts from Dr. P. M. Howley (Harvard University). pCI1E12 was a gift from Dr. M. R. Botchan (University of California, Berkeley, CA). pCGER2Nco (also known as pCG-18E2) was a gift from Dr. C. Dermeret (Pasteur Institute). pRcCMV6bE2 was made by inserting the EcoRI-XhoI fragment encoding the E2 open reading frame of HPV-6b using appropriate polymerase chain reaction primer. pEBG-18E2, an expression plasmid for glutathione S-transferase (GST)-fused HPV-18 E2 protein, was constructed by inserting the BamHI-NcoI fragment encoding the E2 open reading frame of HPV-18 into pEBG vector using the appropriate polymerase chain reaction primer. pEBG-18E2, an expression plasmid for glutathione S-transferase (GST)-fused HPV-18 E2 protein, was constructed by inserting the BamHI-NcoI fragment encoding the E2 open reading frame of HPV-18 into pEBG vector using the appropriate polymerase chain reaction primer. pEBG-18E2, an expression plasmid for glutathione S-transferase (GST)-fused HPV-18 E2 protein, was constructed by inserting the BamHI-NcoI fragment encoding the E2 open reading frame of HPV-18 into pEBG vector using the appropriate polymerase chain reaction primer.

**Design of Plasmid Mutants**—The pcDNA3-HA-mCBP and pcG-18E2 were pelleted, immunoprecipitated, boiled for 10 min, separated on SDS-polyacrylamide gel, and electroblotted onto nitrocellulose membranes. The blots were blocked and hybridized with either anti-HA antibody or anti-E2 antibody. Proteins were detected by chemiluminescence (ECL, Amersham Pharmacia Biotech).

**GST Pulldown Assay**—To determine whether HPV-18 E2 interacts physically with CBP and its homolog p300, we carried out a mammalian two-hybrid assay (CLONTECH) in C33A cells with GAL4-CBP or GAL4-p300 as the target (Fig. 1). Two chimeric proteins were created by fusing full-length CBP or p300 in-frame to the DNA binding domain of GAL4 (GAL4-CBP and GAL4-p300, respectively) and by fusing full-length HPV-18 E2 to the transcriptional activation domain of VP16 (VP16–18E2). Fig. 1B shows that VP16–18E2 activates transcription via the GAL4-CBP chimeric protein. VP16–18E2 also activated transcription via GAL4-p300, indicating that HPV-18 E2 binds to CBP and p300 in a similar manner.

In order to demonstrate the association of HPV-18 E2 with CBP in vivo, co-immunoprecipitation of two proteins was carried out as shown in Fig. 2. A plasmid expressing GST-18E2 protein was transiently co-transfected with a CBP expression plasmid into C33A cells. Negative controls included cells transfected with GST expression plasmid with or without HA-CBP expression plasmid. Selective precipitation of GST protein or HA-tagged protein from the cell lysate showed the co-precipitation of HA-CBP protein (Fig. 2A) or E2 protein (Fig. 2B), confirming that HPV-18 E2 and CBP indeed associate in mammalian cells. To decipher whether HAT activity is associated with a transcription factor E2, we carried out an IP-HAT assay in C33A cells. GST or GST-18E2 expression plasmid was co-transfected into C33A cells. Thirty-six hours after transfection, total cell extract was prepared and GST-tagged proteins were isolated using glutathione-Sepharose 4B beads. Eluates from GST or GST-E2 resin were tested for HAT activity confirming that HPV-18 E2 protein and CBP indeed associate.

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**Results**

CBP Binds to HPV-18 E2—A previous report showed that the NH2 terminus of E2 activates a heterologous promoter in CV1 cells (25). Because CBP is a known co-activator of various transcription factors, we speculated that the mechanism of action of HPV E2, a replication/transcription factor, might involve protein-protein interactions between common cellular transcription factors such as CBP. To provide evidence that E2 interacts physically with CBP and its homolog p300, we carried out a mammalian two-hybrid assay (CLONTECH) in C33A cells with GAL4-CBP or GAL4-p300 as the target (Fig. 1). Two chimeric proteins were created by fusing full-length CBP or p300 in-frame to the DNA binding domain of GAL4 (GAL4-CBP and GAL4-p300, respectively) and by fusing full-length HPV-18 E2 to the transcriptional activation domain of VP16 (VP16–18E2). Fig. 1B shows that VP16–18E2 activates transcription via the GAL4-CBP chimeric protein. VP16–18E2 also activated transcription via GAL4-p300, indicating that HPV-18 E2 binds to CBP and p300 in a similar manner.

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In vivo binding reactions using GST fusion proteins encoding various domains of CBP were performed to characterize...
The translated E2 binds to GST-CBP1 and its derivatives but not to GST-CBP. It was, however, unexpected that E2 could bind to GST-p300. As expected, E2 interacted with specifically this region of amino acids 461–589 is highly conserved between CBP and amino acids 590–669 of CBP. The region of CBP corresponding to 129 (amino acids 461–589) of CBP) and GST-CBP1/80 (amino acids 461–589) of CBP was individually fused to GST. E2 bound specifically to CBP binding reactions (Fig. 3A). A domain of CBP between amino acid residues 450 and 700 has previously been identified as the principle region of CBP required for stable interaction with CREB (2, 19). This region of CBP is referred to as the kinase-induced domain interacting (KIX) domain. We next examined whether the KIX domain is also necessary for CBP/p300 binding to E2. Fig. 3B shows that γS-labeled, in vitro translated E2 binds to GST-CBP1 and its derivatives but not to GST protein. The in vitro translated E2 bound to both GST-CBP1/129 (amino acids 461–589 of CBP) and GST-CBP1/80 (amino acids 590–669 of CBP). The region of CBP corresponding to amino acids 461–589 is highly conserved between CBP and p300. As expected, E2 interacted with specifically this region of CBP. It was, however, unexpected that E2 could bind to GST-CBP1/129. Dai et al. (3) reported that CREB and c-Myb do not bind to this 129-amino acid region (amino acids 461–589) of GST-CBP1. This region contains a glutamine-rich stretch. However, a previous report demonstrated that E2 interacts specifically with Sp1, which contains multiple glutamine-rich domains (32). We propose that E2 has some affinity for glutamine-rich regions. This possibility is currently under investigation.

HPV E2 Interacts with the KIX Domain of CBP through the E2 Transactivation Domain Containing an Amphipathic Helix—To decipher the CBP binding domain within E2, we divided the E2 gene into various fragments and determined which domain of the E2 protein bound to CBP in vivo. This was accomplished using a mammalian two-hybrid assay and measuring the amount of luciferase activity in C33A cells. We hypothesized that the NH₂ terminus of E2 (amino acids 1–226) might contain the CBP binding site, as this region contains a transcriptional activation domain. Table I shows that wild type E2 as well as deletion mutants lacking the E2 DNA binding domain interacted specifically with full-length CBP. Deletion mutants within the E2 amphipathic helix (amino acids 1–155) prevented the specific interaction of E2 with CBP. Interestingly, these deletion mutants showed no intrinsic transactivation properties, whereas full-length E2 or the amphipathic helix motif alone showed a strong activation of luciferase activity. We also observed that the amphipathic helix domain alone interacted very weakly with CBP in the yeast two-hybrid assay (data not shown).

Having established that the KIX domain of CBP can interact directly with HPV E2 in vitro, we designed experiments to test whether this interaction is required and is sufficient for stimulation of transcription by E2 in vivo. The E2 proteins from various HPV strains were tested in this assay. HPV strains fall into two categories: a high risk group (HPV-16 and HPV-18) and a low risk group (HPV-6 and HPV-11) for the development of cervical cancer. As shown in Fig. 4, co-transfection of expression vectors encoding HPV E2 (transcription of HPV E2 was driven by the cytomegalovirus (CMV) immediate early (IE) promoter) and VP16-KIX had a stimulatory effect on 6xE2BStkCAT, which contains six E2BSs in the promoter re-
papillomaviruses. Host cell CBP appears to be a universal property of human CBP and inhibits CREB-dependent transcriptional activation (34). We therefore examined the effects of E1A on E2-dependent transcription. As shown in Fig. 5, E2-dependent transcription was activated by co-transfection of the CBP expression plasmid. These results suggest that overexpression of CBP increases E2-dependent transcription.

CBP binds to a number of cellular and viral regulatory factors, including the adenovirus oncoprotein E1A, which binds to CBP and inhibits CREB-dependent transcriptional activation (34). We therefore examined the effects of E1A on E2-dependent transcription in order to confirm whether CBP is required for E2-dependent transcription. As shown in Fig. 5B, co-transfection of 6xEx2StkCAT with increasing amounts of an E1A expression vector encoding one of four types of HPV E2 (CMV IE promoter-driven) and an expression vector encoding the VP16 activation domain alone (VP16) or a VP16-KIX fusion protein. CAT activity was determined by liquid scintillation counter assay (Promega). Representative results of three experiments are shown.

Fig. 3. Identification of domain of CBP required for binding to E2. A, top, schematic representation of CBP and its functional domains. Shown are the GST-CBP fusion proteins used in GST pull-down assays (see under “Experimental Procedures”). KIX, kinase-induced domain interacting domain; C/H, cysteine/histidine-rich domain; Q-rich, glutamine-rich domain. Bottom, direct interaction of GST-CBP1 with E2 protein in vitro. In vitro translated and 35S-labeled full-length E2 products were incubated with GST alone or with GST-CBP1, GST-CBP2, or GST-CBP3 immobilized on glutathione beads. Labeled proteins (arrows) retained on the beads after extensive washing were analyzed by SDS-PAGE and autoradiography, along with 10% of the translated products used in each incubation (Input). B, top, schematic presentation of GST-CBP fusion proteins used in GST pull-down assays (see the legend to Fig. 2A, top). Bottom, direct interaction of KIX domain with E2 protein in vitro. In vitro translated, 35S-labeled full-length E2 products were incubated with GST alone or with GST-CBP1, GST-CBP1-208, GST-CBP1-129, or GST-CBP1-80 immobilized on the glutathione beads. Labeled proteins (arrows) retained on the beads after extensive washing were analyzed by SDS-PAGE and autoradiography, along with 20% of the translated products used in each incubation (Input).

Fig. 4. Interaction between CBP and E2 from high risk (HPV-16, HPV-18) and low risk (HPV-6b, HPV-11) group HPVs. C33A cells were cotransfected with 1 ng of p6xEx2StkCAT (reporter), 1 ng of an expression vector encoding one of four types of HPV E2 (CMV IE promoter-driven) and an expression vector encoding the VP16 activation domain alone (VP16) or a VP16-KIX fusion protein. CAT activity was determined by liquid scintillation counter assay (Promega). Representative results of three experiments are shown.

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E2 NH2 terminus interacts specifically with CBP in C33A cells

| VP 16 fusion | GAL4 fusion | Binding* |
|--------------|-------------|----------|
| Vector alone | Vector alone | –        |
| E2 (1–155)   | Vector alone | –        |
| E2 (1–246)   | Vector alone | –        |
| E2 (116–365) | Vector alone | –        |
| E2 (261–365) | Vector alone | –        |
| E2 (1–155)   | CBP         | +        |
| E2 (1–246)   | CBP         | +        |
| E2 (116–365) | CBP         | –        |
| E2 (261–365) | CBP         | +        |
| E2 (1–155)   | CBP         | –        |

*In vivo binding was determined with a mammalian two-hybrid assay in C33A cells. The C33A cells were cotransfected with E2-VP16 and CBP-GAL4 expression vectors, along with the pFR-Luc reporter plasmid bearing five GAL4 binding sites. “Vector alone” represents an expression plasmid containing only the VP16 transcriptional activator domain. The numbers after E2 indicate the amino acids of E2 in the VP16-E2 fusion protein. + indicates stimulation of the reporter as compared with results obtained with the reporter and GAL4 expression vector alone (vector alone; contains only to GAL4 DNA binding domain, with no CBP sequence). – indicates basal activity or an activity equivalent to the reporter compared to GAL expression vector alone.
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Fig. 5. CBP activates E2-dependent transcription. A, stimulation of E2-dependent transcription. C33A cells were co-transfected with the p6x2BStkCAT reporter plasmid (1 μg), an E2 expression plasmid (pCG-18E2) (1 μg), and increasing amounts of an expression vector encoding full-length CBP (pCDNA3-HA-mCBP). B, overexpression of CBP in C33A cells can rescue 12S E1A-mediated inhibition of E2 transcriptional activation. C33A cells were transfected with 1 μg of the p6x2BStkCAT reporter plasmid and increasing amounts of either the E1A expression vector (pCDNA3-HA) or the CBP expression vector (pCDNA3-HA-mCBP) with or without 0.5 μg of the E2 expression vector (pCG-18E2). Transcriptional activation was measured using a CAT assay. C, a mutant version of E1A, E1AΔ(Δ2-36), which is unable to bind to p300/CBP, did not perturb E2-dependent transcriptional activation. C33A cells were transfected with p6x2BStkCAT (1 μg) and increasing amounts of the mutant E1A expression vector (pCDNA3-E1AΔ(Δ2-36)) with or without pCG-18E2 (0.5 μg). Transcriptional activation was measured using a CAT assay.

Fig. 6. Transcription-defective E2 mutants show decreased affinity for CBP in vitro. A, schematic diagram of the relative locations of the amphipathic helix motifs and of mutations introduced into E2 in this study. B, transcriptional activities of CBP binding-defective mutant versions of E2. Co-transfection experiments were conducted in C33A cells as described under “Experimental Procedures.” C, association of GST-CBP with E2 wild type protein or E2 mutant proteins. GST pulldown experiments were performed as described under “Experimental Procedures.”

E2-CBP Association Correlates with E2 Activation of Transcription—The relationship between CBP interaction and HPV-18 E2 transcriptional activation was characterized further by comparing wild type E2 function with that of E2 proteins carrying mutations in the region we had defined as the CBP binding domain of E2. In an attempt to generate functional variants of E2, we constructed alanine substitution mutations in the amphipathic helix of E2 (Fig. 6A). Previous reports showed that mutations in this region allowed separation of the transcription and replication functions of E2 for papillomaviruses (37–41). We selected several mutants versions of E2 that were known to be defective for transcriptional activation or DNA replication in PV and generated the corresponding mutated forms of HPV-18 E2. All of the mutant E2 proteins we produced showed reduced transcriptional activation in C33A cells (Fig. 6B). The E39A mutant in HPV-16 E2, which is equivalent to the E43A mutant in HPV-18 E2, retains DNA binding activity and transcriptional activity but loses the ability to stimulate PV DNA replication (39, 40). In our study, the E43A mutant version of HPV-18 E2 showed reduced transcriptional activation and a weak interaction with CBP (Fig. 6, B and C). Other mutants also demonstrated reduced binding affinities for CBP. This correlation of reduced transcriptional activity and loss of CBP interaction highlights the important role of CBP in E2-dependent transcription.

It is possible that CBP is also involved in PV DNA replication, which is mediated by E1 and E2. E1 weakly binds to CBP in an yeast two-hybrid assay (data not shown). This result suggests that E1, E2, and CBP may form a triprotein complex in vivo, and it is possible that these protein-protein interactions are important in PV transcription and/or DNA replication. Several HPV-18 E2 mutants (E6A, I77A, and E78A) that lost the ability to activate PV transcription were able to bind to the KIX domain of CBP in vitro, indicating that CBP association with E2 is not sufficient for transcriptional activation. In addition, we determined whether mutant versions of E2 that are impaired with respect to their ability to bind to CBP could still carry out the cell growth inhibition activity of E2 that is the ability of E2 to inhibit cellular DNA replication. At least two mutant versions of E2 (E43A and I77A) showed defective cell growth inhibition phenotypes when compared with wild type E2 in HeLa cells (data not shown).

CBP HAT Activity Is Necessary for E2-dependent Transcrip-
The E2 proteins from various PVs have a conserved transactivation domain that communicates with host transcription and replication factors. In this study, we demonstrated that the HPV-18 E2 binds to CBP and p300 in vivo and in vitro using co-immunoprecipitation method and GST pulldown assay. We also showed that CBP activates E2-dependent transcription. We further provide evidence that the amphipathic helix regions of E2 play a key role in the E2-CBP interaction. The crystal structure of HPV-18 E2 activation domain showed that NH2-terminal α-helix of E2 draws similarities with the activation domain of p53, CREB, and VP16 (39). Like CREB, E2 interacts with the KIX domain of CBP. Many other cellular transcription factors must communicate with the KIX domain to carry out their transactivation function (1, 2, 3, 5, 44). Although the sequences of these CBP interaction domains share little similarity with each other, all are predicted to form amphipathic helices (45). It has been hypothesized that the KIX domain evolved to recognize diverse partners through its hydrophobic patch. Indeed, E2 indeed has an amphipathic helix able to interact with the KIX domain, and point mutations within this domain result in replicative- or transcription-defective mutants. These results imply that the amphipathic helix of E2 is important for protein-protein interaction with CBP. Recently, two reports showed that HPV-16 E6 directly binds to CBP and represses p53-dependent transcription through protein-protein interaction (46, 47). They demonstrated that HPV-16 E6 binds to C/H1, C/H3, and the COOH terminus of p300/CBP; however, it does not bind to KIX domain of p300/CBP. In our studies, HPV-18 E2 specifically binds to KIX domain of CBP. Therefore, the HPV-18 E2 binding site to CBP does not overlap with the binding site of HPV E6 to CBP. We suggest that the binding of PV transcription/replication factor E2 to KIX domain of CBP is required for transcriptional activation of E2-dependent transcription.

It is known that CBP participates in preventing the G0/G1 transition during the cell cycle by activating certain enhancers and stimulating differentiation pathways. It has also been shown that the transactivation function of CBP is required for E2-mediated growth arrest in HeLa cells (16, 17, 30). The requirement for E2-mediated transcriptional activation in the growth arrest of PV-infected cells suggests that the E2 protein cause growth inhibition by interacting with cellular transcriptional regulatory proteins that participate in proliferation inhibited by interaction with E2 and/or growth arrest activated by interaction with E2. Such results imply that the E2-CBP interaction may modulate the host cell cycle by affecting functional interactions between CBP and other cellular factors. It is also possible that E2 directly binds to a number of host transcription factors and synergistically activates cellular promoters involved in cell growth inhibition by recruiting CBP or other co-activators.

The regulation of chromatin structure plays an important role in the regulation of transcription in eucaryotes and in the initiation of DNA replication from a nucleosomal origin (48, 49). Biochemical and genetic studies have identified various macromolecular complexes that affect chromatin structure to facilitate the interaction of transcription factors with their cognate DNA regulatory elements (50–54). Such complexes include the SWI-SNF complex, nucleosome remodeling factor, chromatin accessibility complex, and the ATP-utilizing chro-
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matin assembly and remodeling factor. Each of these complexes contains an ATPase that is stimulated by naked DNA or nucleosomal DNA (reviewed in Ref. 49). It is probable that these ATPases are the engine of the chromatin remodeling machinery of the cell. Previous genetic studies from yeast have implied that there is functional overlap between chromatin remodeling machinery and HAT activity (55–57). This suggests that HAT activity of CBP as well as the chromatin remodeling machinery may contribute to PV transcription and DNA replication (58, 59). On the basis of our results, we speculate that viral DNA-binding protein E2, along with replication initiator machinery may contribute to PV transcription and DNA replication preinitiation complexes at specific sites in the PV genome. These complexes are likely to contain basal transcription/replication machinery, chromatin remodeling complexes, and transcriptional co-activator complexes. Recently, we showed that hSNF5, a component of SWI-SNF complex, binds to HPV-18 E1 and stimulates HPV DNA replication (59). Through specific, regulated interactions with a number of cellular components, such as the SWI-SNF macromolecular complex, E2 may modulate cellular gene expression at specific stages of the virus life cycle.

Although E2 mutants were inactive for transcriptional activation, they could still stimulate p53-dependent transactivation to a certain extend in HeLa cells (60). We showed that these two E2 proteins (E43A and I77A) were weakly bound to CBP in a GST pulldown assay (Fig. 6). One possible explanation is that E2-CBP association may be contributed to p53-dependent transcription using a stabilizing p53-CBP complex or by an unknown mechanism. These possibilities would need further study.

In this study, we demonstrated the protein-protein interaction between HPV E2 and p300/ CBP and that the HAT activity of CBP is involved in HPV E2-dependent transcription. Additional HAT complexes and histone deacetylase complexes may be required for optimal and selective viral and cellular transcription and DNA replication. Study of the HPV E2 protein will provide important information on the involvement of chromatin remodeling and co-activator complexes in mammalian transcription and DNA replication.

REFERENCES

1. Arias, J., Alberts, A. S., Brindle, P., Claret, F. X., Smeal, T., Karin, M., Feramisco, J., and Montminy, M. (1994) Nature 370, 226–229
2. Chiv Oasis, J., C., Kwok, R. P., Lamb, N., Hagiwara, M., Montminy, M. R., and Goodman, R. H. (1995) Nature 374, 585–589
3. Dai, P., Akimaru, H., Tanaka, Y., Hou, D. X., Yasukawa, T., Kanie-Ishi, C., Takahashi, T., and Ishii, S. (1996) Genes Dev. 10, 528–540
4. Gerritsen, M. E., Williams, A. J., Neish, A. S., Mohr, S., Shy, Y., and Collins, T. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 2927–2932
5. Bannister, A. J., and Kouzarides, T. (1996) Nature 384, 641–644
6. Chen, H., Lin, R. J., Schilt, R. L., Chakravarti, D., Nash, A., Nagy, L., Privalsky, M. L., Nakatani, Y., and Evans, R. M. (1997) Cell 90, 569–580
7. Ogryzko, V. V., Schiltz, R. L., Russanova, V., Howard, B., and Nakatani, Y. (1996) Cell 87, 953–959
8. Spencer, T. E., Jenster, G., Burcin, M. M., Allis, C. D., Zhou, J., Mizzen, C. A., McKennan, N. J., Onate, S. A., Tsai, S. Y., Tsai, M.-J., and O’Malley, B. W. (1997) Nature 389, 194–198
9. Yang, X. J., Ogryzko, V. V., Nishikawa, J., Howard, B. H., and Nakatani, Y. (1996) Nature 382, 319–324
10. Burgering, B. M., Pronk, G. J., van Weeren, P. C., Chardin, P., and Bos, J. L. (1995) EMBO J. 14, 4211–4220
11. Eckner, R., Arany, Z., Ewen, M., Sellers, W., and Livingston, D. M. (1994) Cold Spring Harbor Symp. Quant. Biol. 59, 85–95
12. Eckner, R., Ludlow, J. W., Lill, N. L., Oldred, E., Arany, Z., Modjtabaieh, N., DeCaprio, J. A., Livingston, D. M., and Morgan, J. A. (1996) Mol. Cell. Biol. 16, 3434–3464
13. Yaciuk, P., Carter, M. C., Pipas, J. M., and Moran, E. (1991) Mol. Cell. Biol. 11,