ultracentrifugation on sucrose density gradients (*42*). The Golgi fraction was collected, diluted with 4 volumes of 87.5 mM KOAc, 1.25 mM $Mg(OAc)_2$, and 20 mM Hepes (pH 7.4) and sedimented at 16,000*g* for 10 min. The membrane pellet was washed with transport buffer [0.25 M sorbitol, 70 mM KOAc, 1 mM $Mg(OAc)_2$, 20 mM Hepes (pH 7.4)], then resuspended in 4 ml of transport buffer plus PIC. The Golgi membranes were divided into 0.1-ml samples, frozen in liquid nitrogen, and stored at −80°C.

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Differential Use of CREB Binding Protein–Coactivator Complexes

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CREB binding protein (CBP) functions as an essential coactivator of transcription factors that are inhibited by the adenovirus early gene product E1A. Transcriptional activation by the signal transducer and activator of transcription–1 (STAT1) protein requires the C/H3 domain in CBP, which is the primary target of E1A inhibition. Here it was found that the C/H3 domain is not required for retinoic acid receptor (RAR) function, nor is it involved in E1A inhibition. Instead, E1A inhibits RAR function by preventing the assembly of CBP–nuclear receptor coactivator complexes, revealing differences in required CBP domains for transcriptional activation by RAR and STAT1.

Analysis of the mechanisms by which the adenovirus E1A oncoprotein inhibits cellular differentiation and promotes dysregulated growth has contributed to the identification and functional characterization of cellular regulatory proteins that include Rb, p107, CBP, and p300 (*1*, *2*). CBP and the related p300 function as coactivator proteins for several transcription factors, including CREB (cyclic AMP response element–binding protein) (*3*), AP-1 (*4*), nuclear receptors (*5*), and STAT proteins (*6*). CBP and p300 interact with regulatory proteins through a series of conserved domains (Fig. 1A), with the cysteine-histidine–rich region (C/H3) (*2*) mediating direct interactions with E1A, STAT1, cFos, p/CAF, and RNA helicase A (*4*, *6*–*8*). Although E1A may inhibit the activities of cFos and STAT1 by competing for this interface, other transcription factors that are inhibited by E1A, such as CREB and nuclear receptors, do not interact with this region. E1A inhibits CREB function by preventing the association of a complex of RNA polymerase II and RNA helicase A with the C/H3-E1A interaction domain (*8*). These observations raise the questions of whether recruitment of complexes containing RNA polymerase II to the C/H3-E1A binding site is a general requirement for CBP and p300 to function as transcriptional coactivators and whether competition for this interaction accounts for the inhibitory effects of E1A on other CBP- and p300-dependent factors such as nuclear receptors.

We compared the ability of E1A to inhibit CBP-stimulated transcriptional activation by RARs and STAT1 (*9*). Complete inhibition of RAR activity required about 10 times the amount of E1A than was required for STAT1, suggesting differential use of the C/H3 domain (Fig. 1B). We therefore examined the ability of wild-type CBP and a CBP mutant lacking the C/H3 domain (CBPΔC/H3) to serve as coactivators of RAR and STAT1. Overexpression of wild-type CBP potentiated STAT1-dependent transcription, whereas CBPΔC/H3 was inactive (Fig. 1B). However, CBPΔC/H3 was nearly as effective as wild-type CBP in stimulating RAR activity (Fig. 1B), indicating that the C/H3 domain is not required for CBP to serve as a coactivator of RAR.

We tested the ability of E1A to inhibit the stimulatory effects of CBPΔC/H3 on RAR-dependent transcription. E1A was as effective in inhibiting the coactivator function of CBPΔC/H3 as it was in inhibiting wild-type CBP (Fig. 2A), and glutathione S-transferase (GST)–E1A fusion proteins interacted with CBPΔC/H3 almost as effectively as with wild-type CBP [Fig. 2B and

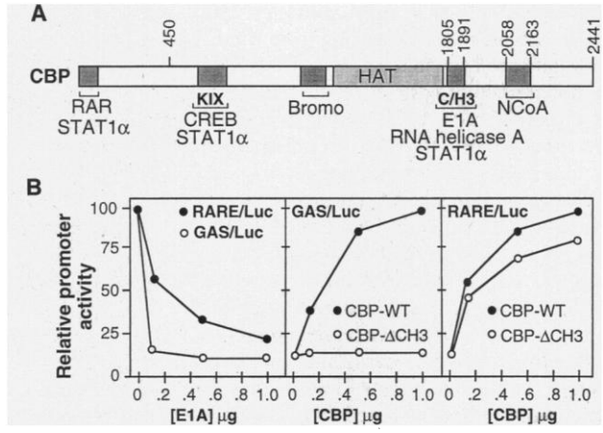

Fig. 1. The retinoic acid receptor (RAR) and STAT1 exhibit differential requirements for the C/H3 domain of CBP. (**A**) CBP interaction domains. The KIX (kinase-inducible interaction) domain mediates interactions with phosphorylated CREB and STAT1. C/H3 is a cysteine-histidine–rich region that mediates interactions with several factors, including E1A, STAT1, and complexes of RNA helicase A and RNA polymerase II. The region in CBP that interacts with nuclear receptor coactivators is indicated as NCoA. Numbers above CBP indicate endpoints of CBP deletion mutants. (**B**) Effects of increasing amounts of 12*S* E1A on CBP-stimulated activities of the RAR (far left) and STAT1 and coactivator functions of wild-type CBP and CBPΔC/H3 molecules for the RAR and STAT1 (middle and far right). HeLa cells were transfected with a luciferase reporter gene under transcriptional control of a minimal promoter linked to two copies of the RARβ2 retinoic acid response element (RARE/Luc) or eight copies of a consensus GAS element recognized by STAT1 (GAS/Luc). Cells were cotransfected with E1A, CBP, or CBPΔC/H3 expression vectors and treated with the retinoic acid–specific ligand TTNPB, interferon-γ, or vehicle, as indicated in (*9*).

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(*10*)], indicating the presence of additional E1A interaction domains within CBP (*11*). To localize the additional E1A interaction domains, a series of CBP and p300 fragments were expressed as GST fusion proteins and assessed for their ability to interact with E1A produced by translation in vitro. The CBP fragment of residues 1459 to 1891 containing the C/H3 domain interacted strongly with E1A. However, interactions of comparable strength were also observed for the NH_2- and COOH-terminal fragments of CBP (Fig. 2C) and p300 (*10*). A series of deletions of the CBP COOH-terminus indicated that a 105–amino acid region between residues 2058 and 2163 was sufficient to mediate interactions with E1A (Fig. 2C). Similar results were obtained with the corresponding region of p300 (*10*). Far Western blotting experiments confirmed that the interactions between E1A and the NH_2- and COOH-terminal domains of CBP were specific and direct (*10*, *11*).

The COOH-terminal E1A interaction domain in CBP and p300 corresponds to the region that mediates interactions with members of the 160-kD family of nuclear receptor coactivators (NCoAs) that associate with the RAR in a ligand-dependent manner and include the structurally related steroid hormone receptor coactivator–1 (SRC-1) and p300/CBP-interacting protein (p/CIP) (*5*, *12*–*14*). p/CIP is required for RAR function and is associated with most of the CBP and p300 in cells (*14*). Increasing concentrations of E1A effectively inhibited the interactions of p/CIP with the CBP NCoA interaction domain (Fig. 3A). E1A did not inhibit other specific protein-protein interactions, and no interaction was observed between E1A and p/CIP (*10*). Therefore, E1A and p/CIP specifically bind to the NCoA interaction domain of CBP in a mutually exclusive manner. To determine whether E1A can inhibit CBP-p/CIP interactions in vivo, we infected P19 cells with a retrovirus directing the expression of 12*S* E1A or a control retrovirus. Immunoprecipitates prepared from cells infected with virus directing expression of E1A exhibited a significant decrease in the relative amounts of CBP that associated with p/CIP (Fig. 2D), indicating that E1A inhibits the formation of p/CIP-CBP coactivator complexes in cells.

To further assess the functional significance of the E1A interaction with the NCoA interaction domain of CBP, we identified E1A mutants that retained this interaction but did not interact with the C/H3 domain. One mutant, in which the amino acid at position 3 was changed from histidine to asparagine (E1A-H3N) (*15*), retained the ability to interact with the NH_2-terminus and NCoA-binding domain of CBP but exhibited a near complete loss of binding activity for the C/H3 domain (Fig. 3B). Next, regions of CBP containing the NH_2-terminal, C/H3, or COOH-terminal E1A binding sites were linked to the GAL4 DNA binding domain, and their activities were assessed on a GAL4-depen-

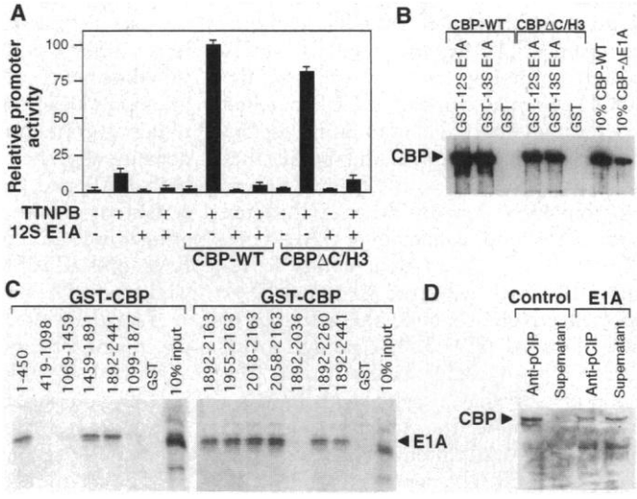

Fig. 2. Identification of two previously unknown E1A interaction domains in CBP. (**A**) E1A inhibits the coactivator function of a CBP molecule lacking the E1A interaction domain. CV1 cells were transfected with the retinoic acid–responsive reporter gene described in Fig. 1 and expression plasmids for E1A, wild-type CBP (CBP-WT), or CBPΔC/H3, as indicated. The labels 12*S*-E1A and 13*S*-E1A designate forms of E1A generated by alternative mRNA processing that either lack (12*S*-E1A) or contain (13*S*-E1A) a COOH-terminal zinc finger domain that is not involved in CBP or p300 interaction. Cells were treated with TTNPB or vehicle for 24 hours before determination of luciferase activity. (**B**) E1A interacts with full-length CBP lacking the E1A interaction domain. Full-length FLAG-tagged CBP or full-length FLAG-tagged CBPΔC/H3 was incubated with GST-12*S* E1A or GST-13*S* E1A bound to glutathione-agarose. See (*11*) for further methods. (**C**) E1A interacts with the NH_2-terminus and distal COOH-terminus of CBP. [^{35}S]12*S* E1A was produced by translation in vitro and incubated with the indicated GST-CBP or GST-p300 fusion proteins. Specifically bound proteins were resolved by SDS-PAGE and detected by autoradiography. Identical results were obtained for 13*S* E1A (*10*). (**D**) E1A inhibits CBP-pCIP interactions in cells. Cells were infected with a retrovirus directing 12*S* E1A expression or a control retrovirus. Whole-cell extracts were prepared from infected cells and subjected to immunoprecipitation with antibodies to p/CIP (anti-p/CIP). The immunoprecipitates or supernatants were resolved by SDS-PAGE and subjected to protein immunoblot analysis with antiserum to CBP.

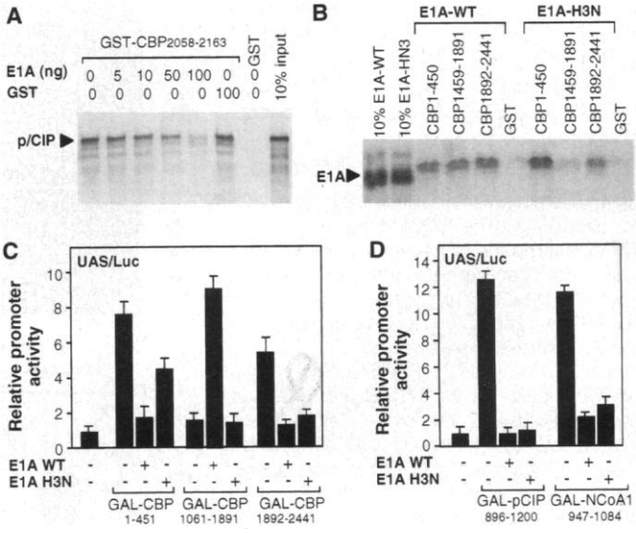

Fig. 3. E1A inhibits the assembly of NCoA-CBP complexes. (**A**) E1A competes with p/CIP for interaction with the COOH-terminus of CBP. GST-CBP(2058–2163) was incubated with [^{35}S]p/CIP produced by translation in vitro and the indicated amounts of purified 12*S* E1A. After washing on glutathione agarose beads, specifically bound [^{35}S]p/CIP was resolved by SDS-PAGE and detected by autoradiography. (**B**) E1A-H3N differentially interacts with the three interaction domains of CBP. [^{35}S]12*S* E1A and [^{35}S]12*S* E1A-H3N were produced by translation in vitro and analyzed for interaction with the indicated GST-CBP fusion proteins as described in Fig. 2. (**C**) Effects of wild-type E1A and E1A-H3N on the transcriptional activities of CBP fragments containing E1A interaction domains. NH_2-terminal, middle, or COOH-terminal regions of CBP containing the three E1A interaction domains were fused to the DNA binding domain of GAL4 and assayed for transcriptional activity on a promoter containing six GAL4 binding sites (UAS/Luc) in the presence or absence of coexpressed wild-type E1A or E1A-H3N. (**D**) Wild-type E1A and E1A-H3N inhibit the p/CIP and NCoA-1 activation domains. Cells were transfected with the UAS/Luc reporter gene and plasmids directing the expression of the GAL4 DNA binding domain linked to the CBP interaction domain of NCoA1 (GAL4-NCoA1 896-1200) or p/CIP (GAL4-p/CIP 947-1084). Cells were cotransfected with 12*S* E1A-WT or 12*S* E1A H3N as indicated and harvested for analysis of luciferase activity 24 hours later.

dent promoter in the absence or presence of coexpressed E1A. The NH_2- and COOH-terminal regions of CBP activated transcription in a manner that was strongly inhibited by wild-type E1A (Fig. 3C), whereas the activity of the C/H3-containing region of CBP was increased. E1A-H3N strongly inhibited the activity of the COOH-terminal domain of CBP but had no effect on the C/H3-containing region. The regions of NCoA-1 and p/CIP that mediate interactions with CBP function as transactivation domains when transferred to the DNA binding domain of GAL4 (*14*), presumably because of their ability to recruit CBP. Both wild-type E1A and E1A-H3N effectively inhibited the activities of these domains (Fig. 3D), consistent with their ability to competitively bind to the region of CBP that interacts with NCoA.

To determine the functional significance of the E1A interaction domains in RAR- and STAT1-dependent transcription, CBP mutants lacking the NH_2-terminal or COOH-terminal E1A interaction domains (CBPΔN450 and CBPΔC1891, respectively) were evaluated for coactivator function. CBPΔN450 was nearly as effective as wild-type CBP as a coactivator of RAR, but not as a coactivator of STAT1 (Fig. 4A). Thus, although the NH_2-terminus of CBP has been demonstrated to interact directly with RAR (5), this interaction does not appear to be required for RAR function. Deletions of the CBP COOH-terminus, containing the NCoA interaction domain, abolished coactivator function for both RAR and STAT1. These results are consistent with the observation that nuclear microinjection of anti-p/CIP blocks both RAR and STAT1 activation (*14*). Next, wild-type E1A and E1A-H3N were evaluated for their effects on RAR- and STAT-dependent activation. E1A-H3N was nearly as effective as wild-type E1A as an inhibitor of RAR function, but much less effective as an inhibitor of STAT1, consistent with the differential requirements of RAR and STAT1 for the C/H3 domain of CBP and p300 (Fig. 4A). E1A molecules containing point mutations or NH_2-terminal deletions that abolish interaction with CBP and p300 (*15*) did not inhibit RAR function (*10*).

To investigate the requirement for the C/H3 domain in activation of endogenous RAR target genes, we examined the effects of wild-type E1A and E1A-H3N on retinoic acid–induced neuronal differentiation of P19 cells (*16*). P19 cells were grown as aggregates and infected with replication-defective retroviruses directing the expression of wild-type E1A or E1A-H3N, or with a virus lacking an E1A insert (*17*). Control cells and virally infected cells were treated with retinoic acid, and differentiation was assessed by both morphology and expression of neurofilament (NF) as a marker of terminal neuronal differentiation. In contrast to uninfected cells or cells infected with control virus, P19 cells that expressed wild-type E1A or E1A-H3N were unable to form either embryoid bodies or cytoplasmic projections and were negative for NF (Fig. 4B). This indicates that the C/H3 domain of CBP and p300 is not required for the inhibitory effects of E1A on retinoic acid–induced differentiation.

Thus, different complexes of CBP or p300 are used by CREB, STAT1, and nuclear receptors (Fig. 4C). Studies of CREB-dependent transcription suggest a requirement for the docking of a complex of RNA polymerase II and RNA helicase A to the C/H3 domain (*8*). The requirement by the C/H3 domain of CBP and p300 for coactivation of STAT1 and the different efficacies of wild-type E1A and E1A-H3N as inhibitors of STAT1 function indicate that the C/H3 domain is the primary target of E1A-mediated inhibition, similar to the case of CREB. In contrast, the C/H3 domain of CBP and p300 is neither required for RAR function, nor is it involved in the mechanism of inhibition by E1A. Differential use of functional domains in CBP and p300 by RAR and STAT1 is consistent with recent findings indicating distinct requirements of these factors for enzymatic and platform assembly functions of CBP- and p300-associated factors (*18*). The finding that E1A inhibits RAR function by preventing the association of CBP with nuclear receptor coactivators raises the possibility that the assembly of these complexes is also subject to regulation by cellular proteins.

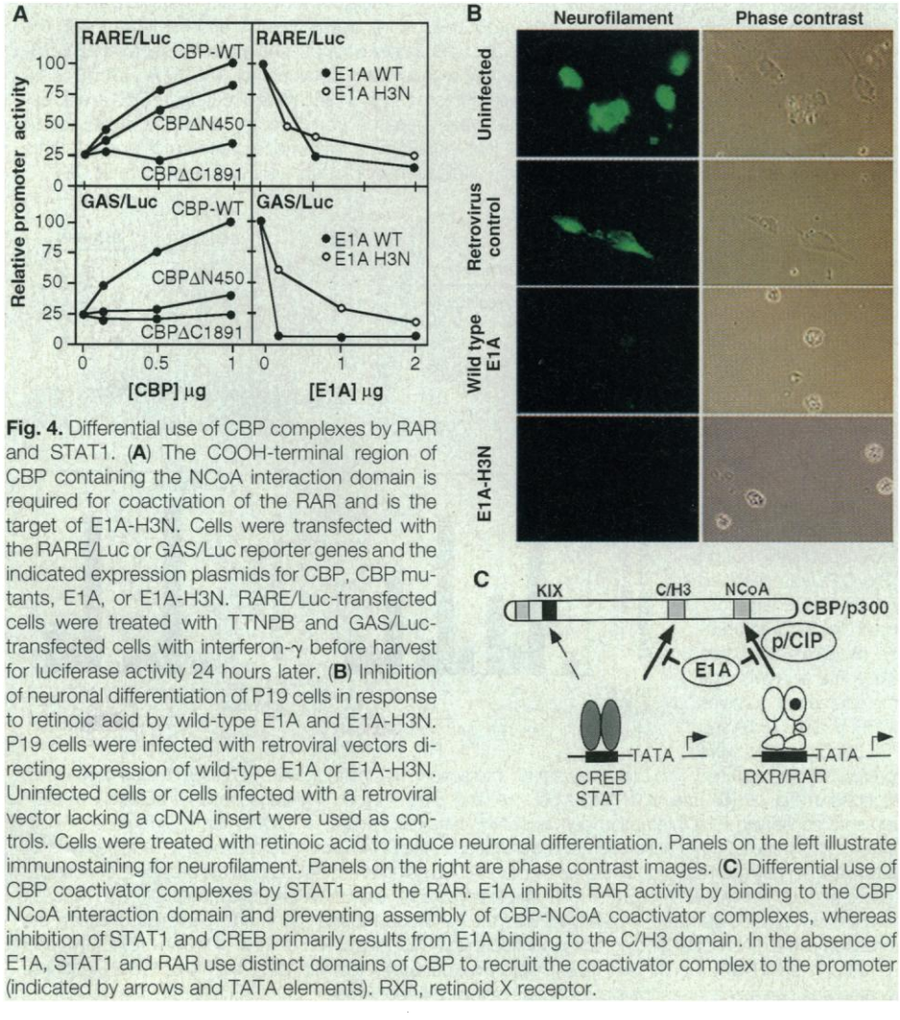

Fig. 4. Differential use of CBP complexes by RAR and STAT1. (**A**) The COOH-terminal region of CBP containing the NCoA interaction domain is required for coactivation of the RAR and is the target of E1A-H3N. Cells were transfected with the RARE/Luc or GAS/Luc reporter genes and the indicated expression plasmids for CBP, CBP mutants, E1A, or E1A-H3N. RARE/Luc-transfected cells were treated with TTNPB and GAS/Luc-transfected cells with interferon-γ before harvest for luciferase activity 24 hours later. (**B**) Inhibition of neuronal differentiation of P19 cells in response to retinoic acid by wild-type E1A and E1A-H3N. P19 cells were infected with retroviral vectors directing expression of wild-type E1A or E1A-H3N. Uninfected cells or cells infected with a retroviral vector lacking a cDNA insert were used as controls. Cells were treated with retinoic acid to induce neuronal differentiation. Panels on the left illustrate immunostaining for neurofilament. Panels on the right are phase contrast images. (**C**) Differential use of CBP coactivator complexes by STAT1 and the RAR. E1A inhibits RAR activity by binding to the CBP NCoA interaction domain and preventing assembly of CBP-NCoA coactivator complexes, whereas inhibition of STAT1 and CREB primarily results from E1A binding to the C/H3 domain. In the absence of E1A, STAT1 and RAR use distinct domains of CBP to recruit the coactivator complex to the promoter (indicated by arrows and TATA elements). RXR, retinoid X receptor.

REFERENCES AND NOTES

1. P. Whyte, N. M. Williamson, E. Harlow, *Cell* **56**, 67(1989); J. R. Nevins, *Science* **258**, 424 (1992); Z. Arany, W. R. Sellers, D. M. Livingston, R. Eckner, *Cell* **77**, 799 (1994); J. R. Lundblad, P. S. Kwok, M. E. Laurance, M. L. Harter, R. H. Goodman, *Nature* **374**, 85 (1995).
2. R. Eckner *et al.*, *Genes Dev.* **8**, 869 (1994).
3. R. P. Kwok *et al.*, *Nature* **370**, 223 (1994).
4. A. J. Bannister and T. Kouzarides, *EMBO J.* **14**, 4758 (1995); J. Arias *et al.*, *Nature* **370**, 226 (1994).
5. D. Chakravarti *et al.*, *Nature* **383**, 99 (1996); B. Han-

stein *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 11540 (1996); Y. Kamei *et al.*, *Cell* **85**, 403 (1996).
6. J. J. Zhang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 15092 (1996); S. Bhattacharya *et al.*, *Nature* **383**, 344 (1996); A. E. Horvai *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **94**, 1074 (1997).
7. X. J. Yang, V. V. Ogryzko, J. Nishikawa, B. H. Howard, Y. Nakatani, *Nature* **382**, 319 (1996).
8. T. Nakajima, C. Uchida, S. F. Anderson, J. D. Parvin, M. Montminy, *Genes Dev.* **11**, 738 (1997); T. Nakajima *et al.*, *Cell* **90**, 1107 (1997).
9. Mutations in CBP and E1A were generated with the polymerase chain reaction. Small regions spanning each deletion or point mutation were confirmed by sequence analysis and transferred into the wild-type cDNA backbone. At least two independent clones of each mutant were analyzed for expression and function. For transient transfection assays of CBP and E1A function, we plated CV1 or HeLa cells in 24-well plates and transfected them with luciferase reporter genes and CBP or E1A expression vectors using $CaPO_4$ (*19*). Salmon sperm DNA was used as a carrier to balance the total amount of transfected DNA. Cells were treated with the RAR-specific ligand TTNPB {(E)-4-[2-(5,5,8,8-tetramethyl-5,6,7,8-tetrahydro-2-napthalenyl)-1-propenyl] benzoic acid} (0.1 μM) or interferon-γ (100 u/ml) for 24 hours or 6 hours, respectively, before determination of luciferase activity.
10. R. Kurokawa and D. Kalafus, unpublished data.
11. Full-length CBP and CBPΔC/H3 were expressed in SF9 cells with a baculovirus vector and contained a COOH-terminal FLAG epitope, which permitted purification on a matrix of FLAG antibody. GST fusion proteins were expressed in *Escherichia coli* and purified on a glutathione agarose affinity matrix. In studies with full-length CBP, SF9 whole-cell extracts containing FLAG-tagged CBP were incubated with GST-E1A fusion proteins produced in bacteria and captured on a glutathione affinity matrix. After they were washed, protein complexes were resolved by SDS–polyacrylamide gel electrophoresis (SDS-PAGE) and analyzed by protein immunoblotting with a monoclonal antibody to FLAG. In studies examining the interaction of p/CIP and E1A with specific domains of CBP, [^{35}S]p/CIP or [^{35}S]E1A proteins were produced by translation in vitro and incubated with GST-CBP fusion proteins. After they were extensively washed, specifically bound proteins were resolved by SDS-PAGE and detected by autoradiography. For Far Western analysis of E1A-CBP interactions, 12*S* E1A and 13*S* E1A cDNAs were introduced into a vector providing an in-frame phosphorylation site for protein kinase A. After phosphorylation with ^{32}P, radiolabeled 12*S* E1A and 13*S* E1A were used to probe nitrocellulose membranes containing CBP fragments as previously described (*12*).
12. S. Halachmi *et al.*, *Science* **264**, 1455 (1994); V. Cavailles, S. Dauvois, P. S. Danielian, M. G. Parker, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 10009 (1994).
13. S. A. Oñate, S. Y. Tsai, M.-J. Tsai, B. W. O'Malley, *Science* **270**, 1354 (1995); J. J. Voegel, M. J. S. Heine, C. Zechel, P. Chambon, H. Gronemeyer, *EMBO J.* **15**, 3667 (1996); T.-P. Yao, G. Ku, N. Zhou, R. Scully, D. M. Livingston, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 10626 (1996); H. Hong, K. Kohli, A. Trivedi, D. L. Johnson, M. R. Stallcup, *ibid.*, p. 4948; H. Chen *et al.*, *Cell* **90**, 569 (1997); S. L. Anzick *et al.*, *Science* **277**, 965 (1997).
14. J. Torchia *et al.*, *Nature* **387**, 677 (1997).
15. H.-G. H. Wang *et al.*, *J. Virol.* **67**, 476 (1993).
16. M. W. McBurney, E. M. V. Jones-Villeneuve, M. K. S. Edwards, P. J. Anderson, *Nature* **299**, 165 (1982).
17. To assess the effects of E1A mutants on retinoic acid–dependent differentiation of P19 cells, we cotransfected human kidney carcinoma 293 cells with 1.5 μg per 6-cm dish of pCL-7 helper-free retroviral packaging vector and pCL1-E1A, pCL1-H3N, and pCL1 vector as indicated, using the $CaPO_4$ method. Media was changed 8 hours after transfection. After 12 hours the supernatant was used in 1:100 dilution for infecting P19 cells. Retroviral vectors and packaging plasmids that produce helper-free retrovirus with 1×10^6 to 5×10^6 titers (*19*) were used for E1A and H3N overexpression. The supernatant of 293 cells infected with pCL-7, pCL1-E1A, or pCL1-H3N packaging constructs were used to infect P19 cells for 2 days. P19 cells were induced to differentiate with 0.5 μM retinoic acid in bacterial-grade petri dishes. Two days after infection and aggregation in the presence of retinoic acid, embryoid bodies were collected and plated again in bacterial-grade petri dishes in the absence of virus. Two days later, cells were transferred to poly-L-lysine–coated glass cover slips in media without retinoic acid (*19*). After 2 to 3 days cells were fixed with 4% formaldehyde and prepared for immunohistochemistry. Rabbit polyclonal antibodies to NF (Sigma) were diluted 1:100 in 0.5% blocking buffer (Boehringer Mannheim). Detection antibody fluorescein isothiocyanate–conjugated to goat antibody to rabbit $F(ab)_2$ fragments was used in 1:100 dilution. Fixed P19 cells were incubated with antibodies to NF for 2 hours at room temperature and rinsed before applying secondary antibody for two more hours at room temperature. Immunofluoresence-stained preparations were viewed with a Zeiss Axiophot photomicroscope. Photographic prints referring to comparative immunostaining were prepared under identical conditions.
18. E. Korzus *et al.*, *Science* **279**, 703 (1998).
19. R. Kurokawa *et al.*, *Nature* **371**, 528 (1994); R. K. Naviaux, E. Costanzi, M. Haas, I. Verma, *J. Virol.* **70**, 5701 (1996).
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Transcription Factor–Specific Requirements for Coactivators and Their Acetyltransferase Functions

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Different classes of mammalian transcription factors—nuclear receptors, cyclic adenosine 3′,5′-monophosphate–regulated enhancer binding protein (CREB), and signal transducer and activator of transcription-1 (STAT-1)—functionally require distinct components of the coactivator complex, including CREB-binding protein (CBP/p300), nuclear receptor coactivators (NCoAs), and p300/CBP-associated factor (p/CAF), based on their platform or assembly properties. Retinoic acid receptor, CREB, and STAT-1 also require different histone acetyltransferase (HAT) activities to activate transcription. Thus, transcription factor–specific differences in configuration and content of the coactivator complex dictate requirements for specific acetyltransferase activities, providing an explanation, at least in part, for the presence of multiple HAT components of the complex.

Nuclear receptors exhibit ligand-dependent interactions with coactivators such as CBP/p300 (*1–3*) and p160 proteins (*4*), steroid receptor coactivator-1 (SRC-1)/NCoA-1 (*2*, *5*), TIF2/GRIP-1/NCoA-2 (*6*), and p300/CBP-interacting protein (p/CIP) (*7*, *8*). CBP/p300 interacts with other coactivators and with the p300/CBP-associated factor (p/CAF) (*9*), which is homologous to the yeast transcriptional adaptor GCN5 (*10*). Both CBP/p300 and p/CAF exhibit strong histone acetyltransferase (HAT) activities (*9*, *11*), whereas the p160 factors possess weak COOH-terminal HAT activity (*8*, *12*).

To determine whether p/CAF is recruited into the nuclear receptor coactivator complex (*2–5*, *13*), we performed coimmunoprecipitation assays with cell extracts. p/CAF exhibited ligand-dependent recruitment to the retinoic acid receptor (RAR) coactivator complex, and p/CAF binding was abolished by binding of an RAR antagonist (Fig. 1A). A minimal ligand-dependent interaction was observed in a yeast two-hybrid assay, and this interaction domain mapped to the NH_2-terminus (amino acids 1 to 351) of p/CAF (Fig. 1B). Using the avidin-biotin complex DNA assay (*14*) to assess protein interactions on DNA-bound receptors, we found that p/CAF bound to the RAR/RXR (retinoid-X receptor) heterodimer, but there was no detectable ligand-dependence for this association (Fig. 1C). The interaction between nuclear receptor and p/CAF was inhibited by the nuclear receptor corepressor (NCoR) (*15*)

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