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Chromatin-Dependent Cooperativity between Constitutive and Inducible Activation Domains in CREB

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The cyclic AMP (cAMP)-responsive factor CREB induces target gene expression via constitutive (Q2) and inducible (KID, for kinase-inducible domain) activation domains that function synergistically in response to cellular signals. KID stimulates transcription via a phospho (Ser133)-dependent interaction with the coactivator paralogs CREB binding protein and p300, whereas Q2 recruits the TFIID complex via a direct association with $hTAF_{II}130$. Here we investigate the mechanism underlying cooperativity between the Q2 domain and KID in CREB by in vitro transcription assay with naked DNA and chromatin templates containing the cAMP-responsive somatostatin promoter. The Q2 domain was highly active on a naked DNA template, and Ser133 phosphorylation had no additional effect on transcriptional initiation in crude extracts. Q2 activity was repressed on a chromatin template, however, and this repression was relieved by the phospho (Ser133) KID-dependent recruitment of p300 histone acetyltransferase activity to the promoter. In chromatin immunoprecipitation assays of NIH 3T3 cells, cAMP-dependent recruitment of p300 to the somatostatin promoter stimulated acetylation of histone H4. Correspondingly, overexpression of hTAFII130 potentiated CREB activity in cells exposed to cAMP, but had no effect on reporter gene expression in unstimulated cells. We propose that cooperativity between the KID and Q2 domains proceeds via a chromatin-dependent mechanism in which recruitment of p300 facilitates subsequent interaction of CREB with TFIID.

Cyclic AMP (cAMP) stimulates cellular gene expression via the protein kinase A (PKA)-mediated phosphorylation of CREB at Ser133 (12). Ser133 phosphorylation, in turn, promotes recruitment of the coactivator paralogs CREB-binding protein (CBP) and p300 via a kinase-inducible domain (KID) in CREB (2, 7). CBP and p300 have intrinsic histone acetyltransferase (HAT) activity (4, 27), which appears to be critical for target gene induction via CREB in CBP-depleted cells (15). p300 HAT activity also appears to be critical in vitro for transcriptional induction on chromatin templates (16–18). Recruitment of p300 to the promoter via VP16 and SP1, for example, induces localized nucleosomal acetylation on histones H3 and H4 (18). Targeted acetylation of nucleosomes over the promoter appears to be necessary but not sufficient for target gene activation; GCN5-mediated acetylation over certain promoters in yeast can be uncoupled from transcriptional induction (19).

In addition to their intrinsic HAT activities, CBP and p300 have been found to associate with functional RNA polymerase II (pol II) holoenzyme complexes (1, 13, 14), and recruitment of such complexes appears to be critical for transcriptional activation. Partially purified fractions of CBP-pol II complexes were found to stimulate transcription from a cAMP-responsive reporter gene, for example (26). The beta interferon (IFN-β) enhanceosome, moreover, has been shown to stimulate transcription via three transcription factors (NF-κB, IRFs, and c-jun/ATF-2 heterodimer) that recruit CBP-pol II complexes to the promoter in response to virus infection (40).

Although recruitment of CBP is essential for target gene activation via the Ser133-phosphorylated KID of CREB, it is not sufficient; a glutamine-rich constitutive activation domain referred to as Q2 is also required (5, 31). The Q2 domain stimulates transcription in vitro via a functional interaction with TFIID (10). Q2 specifically associates with the $hTAF_{II}130$ (also referred to as $hTAF_{II}135$) (22) component of TFIID but not TATA binding protein (TBP) or other TBP-associated factors (TAFs) (9, 10, 26, 33, 34). In this regard, Q2 appears to behave similarly to the glutamine-rich B domain of SP1 (11, 34). Comparative studies of the two activators have revealed a conserved sequence motif (LQTL) that mediates complex formation with $hTAF_{II}130$ (11).

The ability of the Q2 domain and KID in CREB to interact with different coactivators has suggested a cooperative mechanism for induction, whereby each domain recruits distinct components of the transcriptional apparatus in response to cAMP stimulus (26). In contrast to these studies, Felinski et al. have recently proposed that transcriptional induction via CREB proceeds exclusively via the Q2 domain without a requirement for CBP-containing complexes (8). Rather, they propose that phospho (Ser133) CREB stimulates CREB activity via an allosteric mechanism that potentiates Q2 activity. The use of naked DNA templates to evaluate CREB activity and TFIID recruitment in these studies (8) could mask potential effects of CBP/p300 on CREB-dependent transcription, however, prompting us to evaluate CREB-dependent tran-

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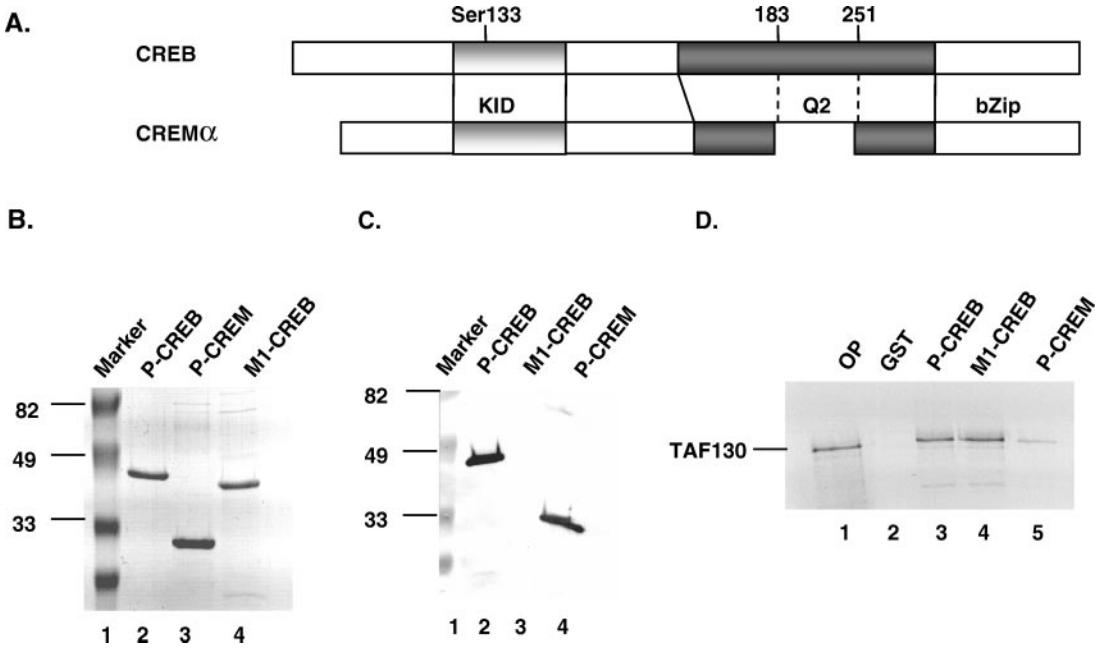


FIG. 1. Phospho (Ser133) KID and Q2 domains in CREB associate with p300 and $hTAF_{II}130$, respectively. (A) Schematic showing the location of the KID and Q2 domain on CREB and CREMα. CREMα lacks residues in Q2 corresponding to aa 183 to 251 of CREB. (B) Coomassie-stained gel of purified recombinant factors employed in in vitro transcription assays. Phospho (Ser133) CREB (lane 2) and phospho (Ser89) CREMα (lane 3) were phosphorylated in vitro with PKA. Recombinant Ser133Ala CREB (M1-CREB) protein is also shown (lane 4). (C) Far-Western blotting assay of recombinant P-CREB, M1-CREB, and P-CREM proteins with alkaline phosphatase-tagged KIX domain polypeptide, which contains aa 591 to 679 of CBP. (D) GST pull-down assay of ^{35}S-labeled $hTAF_{II}130$ with GST only (lane 2), GST M1 CREB (lane 4), GST phospho (Ser133) CREB (lane 3), and GST phospho (Ser89) CREMα (lane 5).

scription on a chromatin template. Our studies confirm the importance of CBP/p300 in this process and provide new insights into the mechanism by which these HATs mediate cooperativity between constitutive and inducible domains in CREB.

MATERIALS AND METHODS

Synthesis and purification of recombinant proteins. His6-tagged rat CREB and CREMα proteins were synthesized in *Escherichia coli* (BL21) and purified by standard techniques. Purified CREB and CREMα proteins were then phosphorylated with PKA. Equal phosphorylation of CREB and CREMα was evaluated by Western blotting with anti-phospho CREB antibody. Comparable CRE binding activity of wild-type, Ser133-phosphorylated, and Ser133A1a mutant CREB proteins was confirmed by gel mobility shift assay. Wild-type, HAT-defective, and ΔC/H3 mutant p300 proteins were expressed in Sf9 cells by using a baculovirus expression system as previously described (17).

Chromatin assembly and in vitro transcription reactions. Wild-type and CRE-mutant somatostatin promoter templates were employed in the in vitro transcription assays (39). Chromatin assembly reactions were performed with a chromatin assembly extract derived from *Drosophila melanogaster* embryos as previously described (17). P-CREB, P-CREM, CREB-M1, and p300 were added after the chromatin assembly reactions were complete. The reaction mixtures were incubated for an additional 30 min at 27°C after the p300 proteins were added to allow for interaction with the chromatin templates. In vitro transcription reactions were performed with HeLa cell nuclear extracts that were prepared as previously described (17). The RNA products from the in vitro transcription reactions were analyzed by primer extension analysis as described previously (39). All reactions were performed in duplicate, and each experiment was performed a minimum of two separate times to ensure reproducibility. The data were analyzed and quantified with a PhosphorImager (Molecular Dynamics).

Transfection assay. To analyze CREB transcription activity in the context of an integrated reporter, NIH 3T3 cell lines, referred to as KD-1 cells, were stably transfected with a GAL4 luciferase gene containing five GAL4 recognition sites upstream of the adenovirus E1B promoter. Transfections were performed as previously described (3). In a typical assay, 0.5 μg of GAL4-CREB (amino acids [aa] 1 to 283), 0.5 μg of wild-type or mutant p300 plasmid, and/or 0.5 μg of $hTAF_{II}130$ were transfected into KD-1 cells with Lipofectamine Plus (Gibco BRL). After 24 h, cells were treated with Forskolin (10 μM) or control vehicle for 4 h. Luciferase activity was assayed, and reporter activities were normalized to activity from a cotransfected Rous sarcoma virus (RSV)–β-galactosidase expression plasmid as previously described (3). Comparable expression levels of GAL4 CREB and p300 wild-type and mutant polypeptides were verified by Western blot assay.

Chromatin immunoprecipitation. NIH 3T3 cells harboring multiple copies of the somatostatin gene, referred to as D5 cells (23), were treated with 10 μM Forskolin or control vehicle for 1 h and then treated with formaldehyde to cross-link protein-DNA complexes (23). Immunoprecipitates of cross-linked complexes were prepared with control or acetyl-H4-specific antibody, which recognizes histone H4 acetylated at Lys5, -8, -12, and -16 (Serotec, Raleigh, N.C.). Immunoprecipitates were treated with proteinase K for 2 h and then incubated at 65°C to release cross-links. DNA was purified by phenol-chloroform extraction and ethanol precipitation. DNA samples were then analyzed with 20 cycles of PCR to amplify somatostatin promoter sequences. Different cycle numbers were employed to ensure linearity of amplification. PCR products were analyzed by 1.5% agarose gel with ethidium bromide, and signal intensity was calculated by Kodak Digital Science (Kodak) and ImageQuant (Molecular Dynamics).

GST pull-down assays. Glutathione *S*-transferase (GST)-$TAF_{II}130$ constructs were prepared from full-length $TAF_{II}130$ (21). GST pull-down assays were performed as reported previously (28, 29).

RESULTS

To confirm the domain requirements for recruitment of CBP/p300 and TFIID complexes via CREB, we performed in vitro interaction assays with purified recombinant phospho (Ser133) CREB (P-CREB), phospho (Ser89) CREMα (P-CREM), and Ser133Ala CREB (M1-CREB) (Fig. 1A and B). In far-Western blotting assays, P-CREM and P-CREB asso-

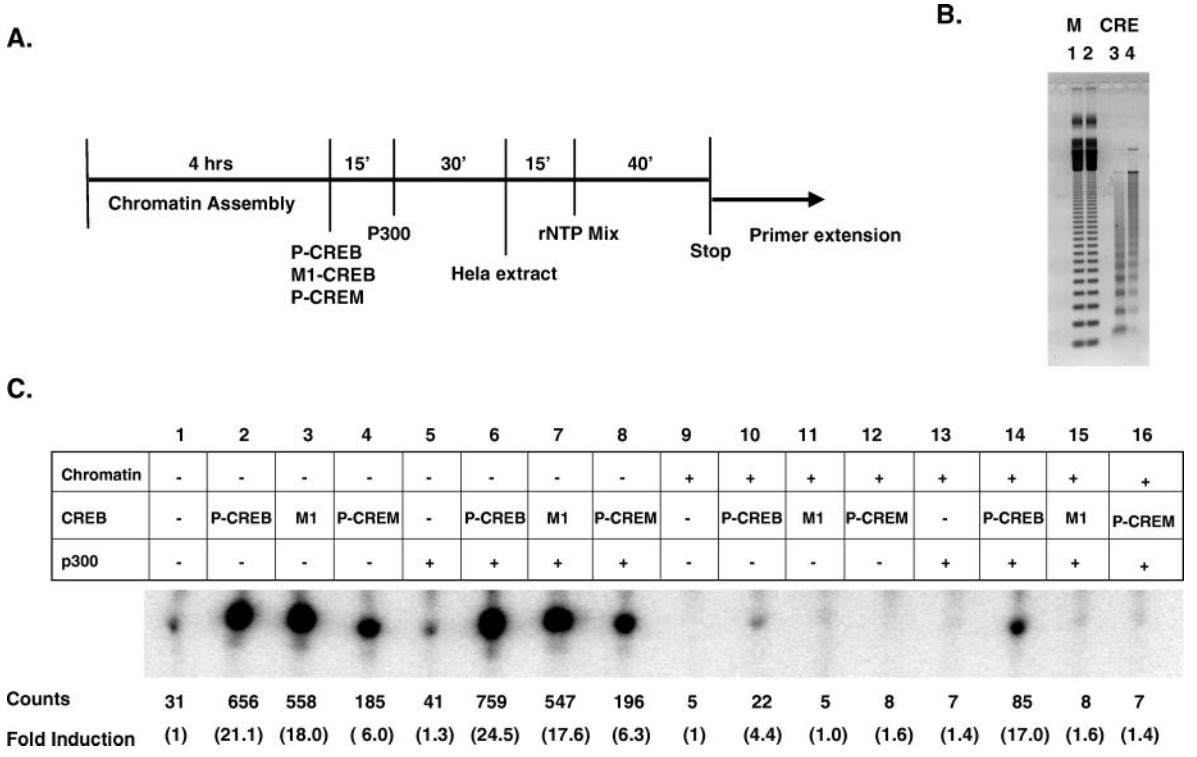


FIG. 2. Cooperativity between the Q2 domain and KID in CREB is chromatin dependent. In vitro transcription assays on naked DNA (lanes 1 to 8) and chromatin templates (lanes 9 to 16) by using the cAMP-responsive somatostatin promoter are shown. (A) The time course of chromatin assembly, nuclear factor addition, and transcription reactions is shown. (B) Micrococcal nuclease digestion of chromatin assembly reaction mixtures demonstrate regularity of nucleosome spacing on the template. M, size marker (lanes 1 and 2). Two concentrations of micrococcal nuclease are shown (lanes 3 and 4). (C) Primer extension assays of in vitro transcription reactions supplemented with purified recombinant p300, phospho (Ser133) CREB (P-CREB), Ser133Ala CREB (M1), or phospho (Ser89) CREM (P-CREM) as indicated. The relative intensity of ^{32}P-labeled primer extension products from individual reactions was determined by phosphorimaging. Fold induction over a control reaction containing no activators (lanes 1 and 9) is indicated. In Fig. 3 and 4, reactions were performed at least twice to confirm reproducibility.

ciated comparably with an alkaline phosphatase-tagged $CBP_{591–679}$ peptide comprising the CREB binding domain, referred to as KIX (32) (Fig. 1C, lanes 2 and 4). Consistent with the previously noted requirement of Ser133 phosphorylation for complex formation with CBP and p300 (7, 28), M1-CREB did not bind detectably to the KIX domain in vitro (Fig. 1C, lane 3).

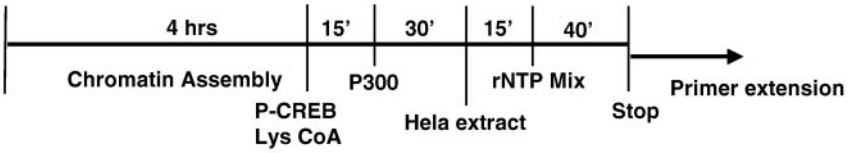
In GST pull-down assays with ^{35}S-labeled $hTAF_{II}130$, both P-CREB and M1-CREB interacted comparably with $hTAF_{II}130$ (Fig. 1D, lanes 3 and 4). P-CREM associated about one-fifth as well with $hTAF_{II}130$, reflecting a 69-aa deletion spanning residues 183 to 251 within Q2 that includes critical residues for interaction with $hTAF_{II}130$ (10) (Fig. 1D, lane 5).

To evaluate the functional basis for cooperativity between KID and the Q2 activation domain, we performed in vitro transcription assays with P-CREB, P-CREM, and M1-CREB proteins (Fig. 2a and b). All three proteins have identical CRE binding activity on the somatostatin promoter (not shown), and their ability to interact with either p300 (P-CREM), $hTAF_{II}130$ (M1-CREB), or both p300 and $hTAF_{II}130$ (P-CREB) allowed us to compare the relative importance of these activities for transcription on naked DNA and chromatin templates.

Addition of phospho (Ser133) CREB to transcription reactions stimulated somatostatin promoter activity 20-fold on a naked DNA template (Fig. 2C, compare lanes 1 and 2). M1-CREB was comparably active to P-CREB on the naked DNA template, suggesting that the phospho (Ser133) KID is dispensable for transcription via CREB in crude nuclear extracts (Fig. 2C, lane 3). Consistent with its relatively weak affinity for $hTAF_{II}130$ in GST pull-down assays (Fig. 1D), P-CREM was only one-fourth as active as P-CREB in stimulating somatostatin transcription on the naked DNA template (Fig. 2C, lane 4). Addition of p300 marginally induced somatostatin promoter activity (1.2-fold) in reaction mixtures containing either P-CREB or P-CREM, having no effect in samples containing M1-CREB (Fig. 2C, lanes 5 to 8). These results support the notion that the Q2 domain can account for most of the transcriptional activity of CREB on a naked DNA template, and that transactivation via p300 is largely undetectable in this system.

To test whether Ser133 phosphorylation of CREB stimulates gene expression via a chromatin-dependent mechanism, we performed in vitro transcription reactions on chromatin templates (Fig. 2C, lanes 9 to 16). P-CREB induced somatostatin transcription fourfold on the chromatin template, but M1-CREB was completely inactive (Fig. 2C, compare lanes 9, 10, and 11), indicating that Q2 domain activity is strongly repressed under these conditions. P-CREM was also inactive on the chromatin template (Fig. 2C, lane 12), demonstrating a requirement for both KID and Q2 domains for transcriptional induction in this context.

A.



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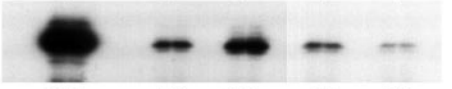
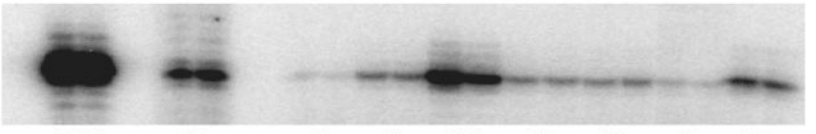


Counts	478	70	190	58	27
Fold Induction		(1)	(2.7)	(0.82)	(0.38)
Chromatin	-	+	+	+	+
P-CREB	+	+	+	+	+
P300	Wt	-	Wt	-HAT	-CH3

C.



Counts	1524	13	5	44	284	35	32	16	60
Fold Induction				(1)	(6.5)	(0.80)	(0.73)	(0.36)	(1.3)
DNA	Wt	-CRE	Wt	Wt	Wt	Wt	Wt	-CRE	Wt
Chromatin	-	-	+	+	+	+	+	+	+
P-CREB	+	+	-	+	+	+	M1	+	+
P300	+	+	-	-	+	-HAT	+	+	+
Lys CoA	-	-	-	-	-	-	-	-	+

FIG. 3. p300 HAT and C/H3 domain activities are required for target gene activation via phospho (Ser133) CREB on a chromatin template. (A) Time course of addition of Lys-CoA, activators, and template. (B) Effect of wild-type, HAT-defective (−HAT), and C/H3 region-deleted (−CH3) p300 polypeptides on transcription from the somatostatin promoter in reaction mixtures containing P-CREB. For panels B and C, duplicate reactions are shown for each condition. Naked DNA and chromatin templates are indicated. Fold induction over reaction mixture lacking p300 (lane 2) is shown in parentheses. (C) Effect of p300 HAT inhibitor Lys-CoA (10 μM) on transcription via P-CREB in vitro. Reactions with wild-type (Wt) and mutant (−CRE) somatostatin promoters containing an inactivating mutation in the consensus CRE site that disrupts binding of CREB are also shown. Fold induction over a reaction mixture lacking p300 (lane 4) is shown.

In contrast to its lack of activity on a naked DNA template, p300 potentiated somatostatin promoter activity fourfold on a chromatin template (Fig. 2C, compare lanes 10 and 14). However, p300 had no effect on somatostatin transcription in reaction mixtures containing M1-CREB, demonstrating a functional requirement for the Ser133-phosphorylated KID under these conditions (Fig. 2C, lane 15). Recruitment of p300 was not sufficient to induce gene expression; transcription from reaction mixtures containing P-CREM remained undetectable even after addition of recombinant p300 (Fig. 2C, lane 16). These results demonstrate the importance of both Q2-$hTAF_{II}130$ and KID-p300 interactions for target gene activation.

To assess the importance of p300 HAT activity for transcription via CREB on a chromatin template, we performed in vitro assays with mutant p300 polypeptides lacking HAT enzymatic activity (Fig. 3A). Compared to the induction observed with wild-type p300, mutant HAT-defective p300 protein containing point mutations in the HAT domain that abolish enzymatic activity (17) had no effect on P-CREB-dependent transcription in vitro (Fig. 3B, compare lanes 2 to 4; Fig. 3C, compare lanes 4 to 6). Addition of the p300-specific HAT inhibitor Lys-coenzyme A (Lys-CoA) (20) also blocked potentiation of CREB activity in vitro (Fig. 3C, lane 9), demonstrating that p300 HAT activity is required for induction of cAMP-responsive genes via phospho (Ser133) CREB. As reported in other studies (18), the time course of addition of Lys-CoA (10 μM) to reactions was critical for inhibition (Fig. 3A); no effect on transcription was noted when Lys-CoA was added to reaction mixtures in conjunction with or after p300 (not shown). These results suggest that p300 HAT activity may only be transiently required for transcriptional induction on a chromatin template.

In addition to its HAT activity, p300 has been proposed to mediate activation of cAMP-responsive genes via a C/H3 do-

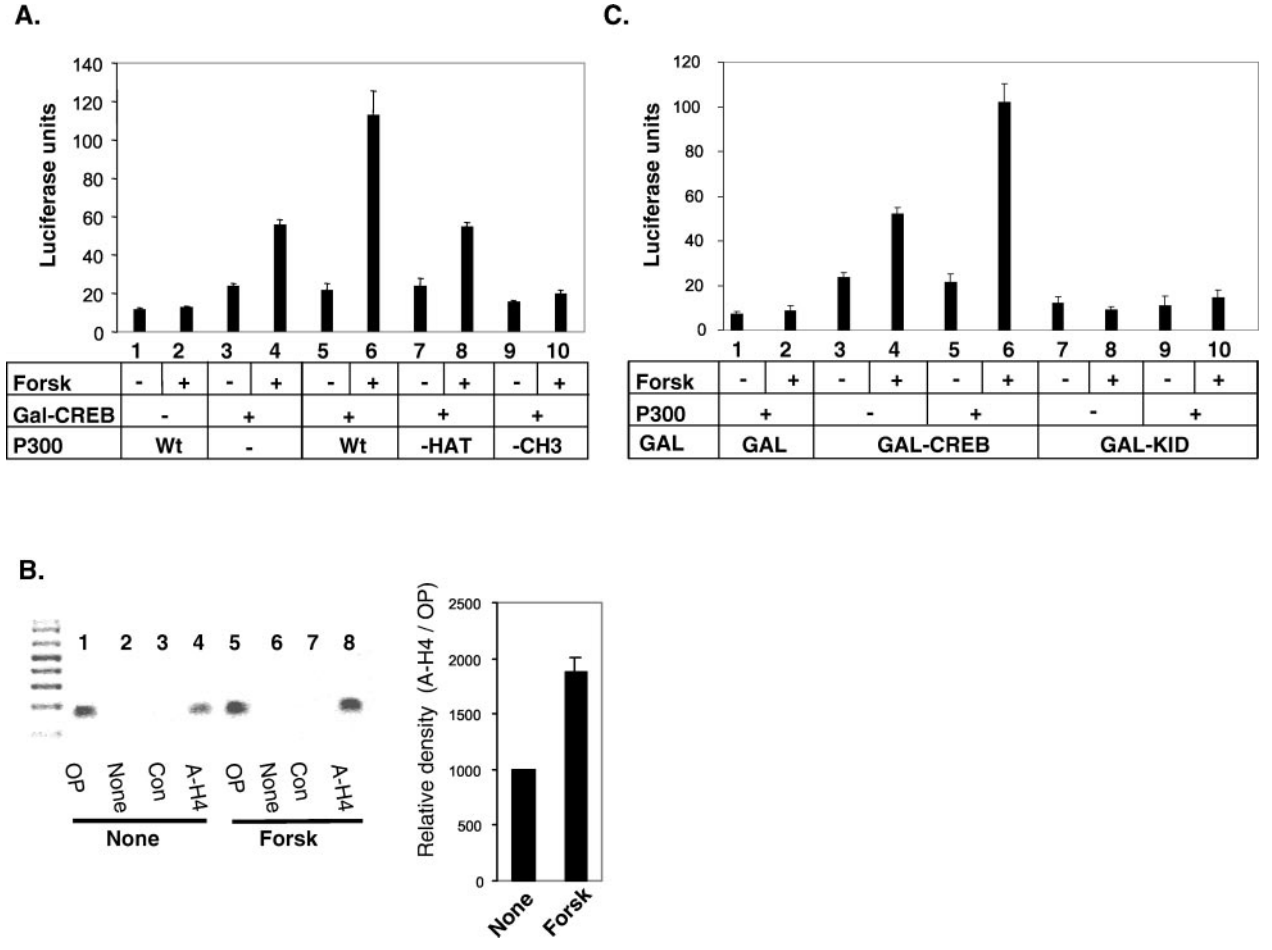


FIG. 4. p300 HAT and C/H3 domain activities are required for cAMP-dependent transcription via CREB in vivo. (A) Transient transfection assay of NIH 3T3 cells harboring integrated copies of a GAL4 luciferase reporter gene that contains four GAL4 recognition sites (KD-1 cells). Cells were transfected with a GAL4 CREB expression vector containing the GAL4 DNA binding domain fused to the N-terminal 283 residues of CREB, which contains both KID and Q2. Cotransfection of wild-type (wt), HAT defective (−HAT), and C/H3 region deletion (−CH3) mutant p300 expression vectors is indicated. Cells were treated with Forskolin (10 μm) or control vehicle as shown. Relative luciferase activity was normalized to β-galactosidase activity from cotransfected RSV β-galactosidase plasmid. Comparable expression of wild-type and mutant p300 polypeptides was verified by Western blot assay (not shown). (B, left) Chromatin immunoprecipitation assay of NIH 3T3 cells containing integrated copies of the rat somatostatin gene (D5 cells). D5 cells were treated with Forskolin (10 μM) or control vehicle for 1 h, and formaldehyde cross-linked protein DNA complexes were immunoprecipitated with anti-acetyl H4 (A-H4) or control antisera (Con). PCR analysis of each immunoprecipitate is shown, including input DNA (OP), as well as a control reaction with no antiserum (none). (Right) Intensity of PCR products estimated by densitometry, as shown in the bar graph. The average of four independent experiments ± standard error is shown. (C) The CREB Q2 domain is required for target gene activation via p300 in KD-1 cells containing integrated copies of a GAL4 luciferase reporter gene. Transfections were performed with p300, GAL4 CREB, GAL4 DNA binding domain (GAL), and GAL4 KID expression vectors. GAL4 KID contains aa 100 to 160 of CREB spanning the KID, which is sufficient for association with p300. Treatment with Forskolin (10 μM, 4 h) is indicated (Forsk).

main-dependent interaction with RNA pol II (26). Compared with wild-type p300, ΔC/H3 p300 protein behaved as a dominant-negative inhibitor of transcription, reducing basal phospho-CREB activity about twofold (Fig. 3B, compare lanes 2, 3, and 5). Notably, wild-type and ΔC/H3 p300 proteins exhibit comparable HAT activities in vitro (17), arguing for a distinct role of this domain compared to the HAT domain in regulating transcription. Taken together, these results demonstrate that both HAT and C/H3 regions of p300 are required for transcriptional activation via CREB in vitro.

To assess the importance of p300 HAT and C/H3 domains for cAMP-dependent target gene induction in vivo, we performed transient transfection assays with a GAL4-CREB expression vector containing the CREB transactivation domain (aa 1 to 283) fused to the GAL4 DNA binding domain. In order to evaluate chromatin-dependent effects of p300 on CREB activity, we isolated stable NIH 3T3 cell clones (referred to as KD-1 cells) harboring chromosomally integrated copies of a GAL4 luciferase reporter plasmid.

Addition of cAMP agonist stimulated the chromosomal GAL4 luciferase reporter threefold in KD-1 cells transfected with GAL4 CREB (Fig. 4A, lanes 1 to 4), and cotransfection with wild-type p300 further potentiated reporter activity twofold in cells exposed to cAMP agonist, but not in control cells (Fig. 4A, lanes 5 and 6). Consistent with in vitro transcription data, HAT-defective p300 had no effect on GAL4 CREB ac-

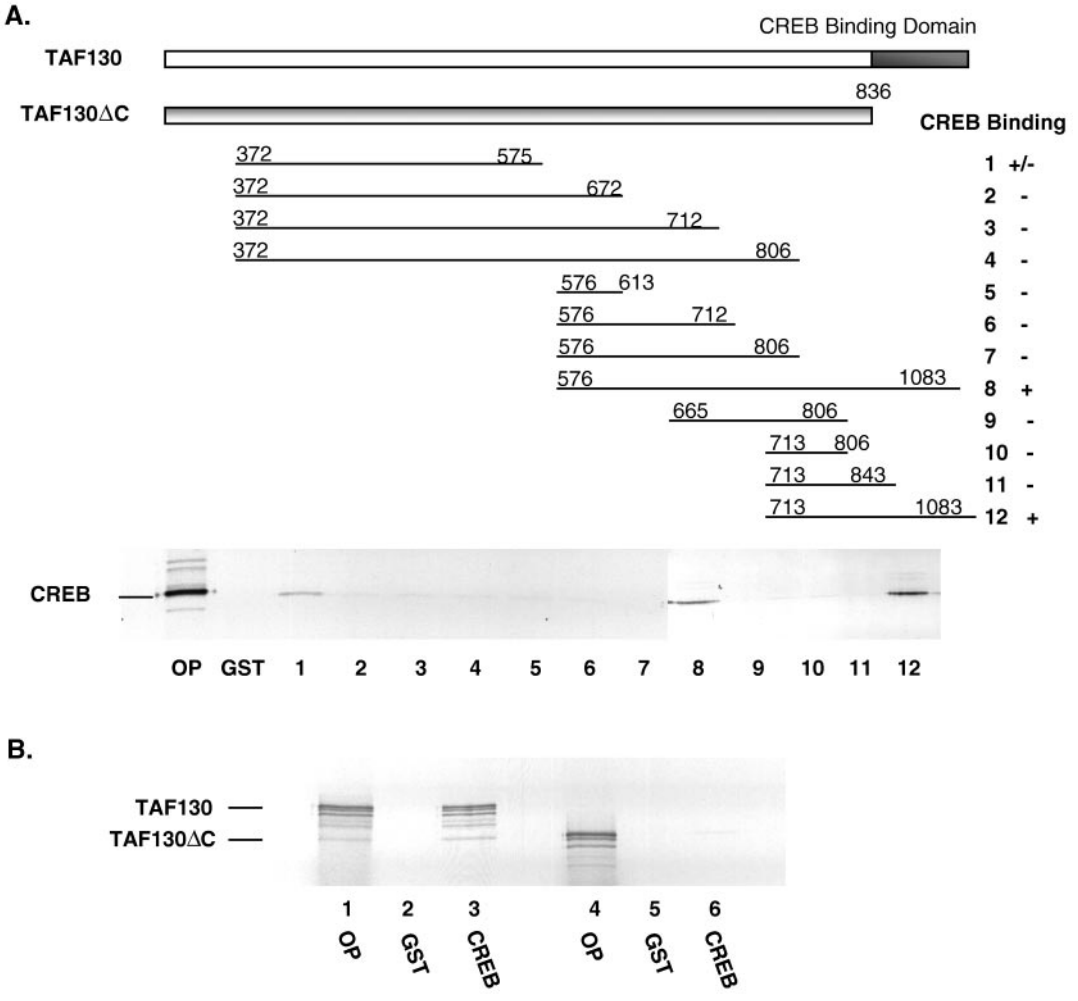


FIG. 5. Conserved region II (CRII, aa 847 to 1083) in $TAF_{II}130$ is required for complex formation with CREB. (A) Pull-down assay of recombinant GST-$TAF_{II}130$ polypeptides with amino acid end points indicated. Interaction with CREB is summarized on the right (+ or −), and corresponding results of the pull-down assay with ^{35}S-labeled full-length CREB protein are shown on the bottom panel. (B) Pull-down assay with ^{35}S-labeled wild-type $TAF_{II}130$ and mutant $TAF_{II}130\Delta C$ lacking the C-terminal CREB binding domain (aa 836 to 1083) with GST-CREB (aa 1 to 283) or GST alone. OP, 10% of input protein.

tivity on the chromosomal template (Fig. 4A, lanes 7 and 8). Similarly, overexpression of mutant p300 lacking the C/H3 domain actually disrupted endogenous p300 activity, reducing GAL4 CREB-dependent transcription threefold following treatment with cAMP (Fig. 4A, compare lanes 3 and 4 and 9 and 10). Taken together, these results confirm the functional importance of p300 HAT and C/H3 domains in promoting target gene activation via CREB on an integrated reporter gene in vivo.

The importance of p300 HAT activity for target gene activation via P-CREB prompted us to test whether recruitment of p300 activity stimulates nucleosome acetylation over the promoter by chromatin immunoprecipitation assays of NIH 3T3 cells expressing integrated copies of the somatostatin gene, referred to as D5 cells (23). Following treatment of D5 cells with forskolin for 30 min, histone H4 acetylation over the somatostatin promoter (−100 to +55) was enhanced 1.5- to 2-fold (Fig. 4B, compare lanes 4 and 8). These results confirm previous observations (23) and suggest that the requirement for p300 HAT activity for CREB transactivation reflects, at least in part, nucleosome acetylation over target promoters in cells exposed to cAMP.

To determine the importance of the KID and Q2 domain for activation on an integrated chromosomal template in vivo, we transfected KD-1 cells with wild-type GAL4 CREB or GAL4 KID effector plasmids. Compared to the two- to threefold induction observed with GAL4 CREB expression vector, GAL4 KID had no effect on luciferase reporter activity in KD-1 cells (Fig. 4C, compare lanes 3 and 4 and 7 and 8). Moreover, cotransfection with p300 potentiated GAL4 CREB, but not GAL4 KID, activity in the presence of cAMP agonist (Fig. 5A, compare lanes 5 and 6 and 9 and 10), supporting the notion that recruitment of p300 HAT activity to the promoter is not sufficient for target gene activation in vivo.

The Q2 domain has been shown to stimulate transcription by recruiting TFIID activity to the promoter via a direct interaction with $TAF_{II}130$ (8, 10, 26). To map the domain of $TAF_{II}130$ that interacts with CREB, we performed GST pull-down assays. Confirming a previous study by using yeast two-hybrid analysis to monitor this association (34), modest binding

of CREB to the central glutamine-rich region of $TAF_{II}130$ was observed (Fig. 5A, lane 1). The carboxy-terminal conserved region II (CRII) of $TAF_{II}130$ appeared to associate with CREB far more efficiently in this regard, however (Fig. 5A, lanes 8 and 12), and deletion of the C-terminal 247 residues(aa 836 to 1038) that comprise this domain inhibited complex formation with CREB in vitro (Fig. 5B, compare lanes 3 and 6).

Consistent with other activator-TAF interactions, such as VP16-$TAF_{II}31$ (38), the CREB-$TAF_{II}130$ interaction is comparatively weak, and complex formation between these proteins in vivo was not detectable by coimmunoprecipitation assay (not shown), prompting us to monitor recruitment of $TAF_{II}130$ by transient assay. Cotransfection of $hTAF_{II}130$ expression plasmid potentiated GAL4 CREB activity 1.5- to 2-fold in KD-1 cells treated with cAMP agonist, but had no effect in untreated cells (Fig. 6A, compare lanes 3 and 4 and 7 and 8). p300 also stimulated GAL4-CREB activity in KD-1 cells exposed to cAMP agonist (Fig. 6A, compare lanes 3 and 4 and 5 and 6), and overexpression of both p300 and $hTAF_{II}130$ vectors further induced reporter activity (Fig. 6A, lanes 9 and 10).

To evaluate the selectivity of $TAF_{II}130$ in stimulating transcription via certain activators, we employed GAL4–c-fos and GAL4–c-jun expression vectors. Although p300 also potentiated target gene expression via GAL4–c-fos (Fig. 6A, lanes 15 to 18) and GAL4–c-jun (not shown), $TAF_{II}130$ had no effect on either polypeptide (Fig. 6A, lanes 19 to 22) (data not shown). Confirming the importance of the CREB-$TAF_{II}130$ interaction target gene induction in this assay, mutant $TAF_{II}130$ polypeptide lacking the CREB-interacting CRII region, designated $TAF_{II}130\Delta C$, did not potentiate GAL4 CREB activity in KD-1 cells (Fig. 6A, lanes 15 to 18). Conversely, GAL4-CREB polypeptide lacking the glutamine-rich Q2 domain was also unresponsive to $TAF_{II}130$ overexpression (Fig. 6B, compare lanes 3 to 6 and 7 to 10). These results support the notion that CREB recruits $TAF_{II}130$ activity to the promoter via an interaction between the Q2 and CRII domains, respectively.

DISCUSSION

CREB has been shown to regulate target gene expression via the Q2 and KID activation domains (6, 31). Our data demonstrate that cooperativity between these domains is chromatin dependent. Thus, the inability of other studies to detect an important function for the phospho (Ser133) KID is consistent with the use of naked DNA templates in these experiments (8).

The cooperativity we observed between the Q2 domain and KID is similar to the combinatorial effects noted between SP1 and SREBP-1 on the low-density-lipoprotein receptor promoter (24). SP1 activates transcription in part via an interaction with $hTAF_{II}130$, whereas SREBP-1 stimulates transcription via a KIX-dependent interaction with p300 (24), paralleling the activities of Q2 domain and KID, respectively. In these studies, SP1–SREBP-1 cooperativity was also shown to be chromatin dependent, although the importance of CBP HAT activity for these effects was not resolved (24). Nevertheless, the conservation of two domain-specific coactivator interactions suggests that this particular mode of combinatorial regulation may favor cooperative assembly of the transcriptional apparatus on the promoter.

In a previous study, we observed cooperative effects of the Q2 domain and KID on transcription from a naked DNA template by using purified fractions containing CBP-pol II and -TFIID complexes (26). However, such cooperativity was not detectable in crude HeLa nuclear extracts (8, 10), suggesting that, at ambient concentrations, CBP-Pol II and TFIID activities are unable to assemble cooperatively on CREB-dependent promoters. Rather, our studies confirm the notion that, like other activators (19), CREB stimulates transcription on naked DNA templates via a p300-independent mechanism (8, 10). Q2 accounts for most of CREB transcriptional activity on a naked DNA template, and Ser133 phosphorylation or p300 addition has no appreciable effect.

The Q2 domain has been shown to promote target gene activation via an association with $TAF_{II}130$ (9, 10, 26, 33, 34). Although a modest interaction between CREB and the glutamine-rich region of $TAF_{II}130$ has been noted in this report and elsewhere (34), a much stronger association was observed with the C-terminal CRII region. In this regard, polyglutamine expansions that are associated with neurodegenerative diseases appear to sequester $TAF_{II}130$ and to suppress CREB activity by binding to CRII (37). By competing for a common interaction domain in $TAF_{II}130$, polyglutamine stretches may thus disrupt CREB-dependent transcription. Importantly, the CRII domain of $TAF_{II}130$ appears to be targeted by a number of nuclear factors, including thyroid hormone receptor (30), progesterone receptor (35), and adenovirus E1A (21), suggesting a common mode of recruitment for this coactivator. Point mutagenesis of the CRII region should reveal whether these proteins recognize common or distinct surfaces in $TAF_{II}130$.

Q2 activity is strongly repressed on a chromatin template, and Ser133 phosphorylation of CREB and p300 recruitment are required for transcriptional induction under these conditions. In the absence of p300, the Q2-$TAF_{II}130$ interaction is apparently too weak to overcome the repressive effects of chromatin. Recruitment of p300 and consequent acetylation of promoter-bound nucleosomes may favor this interaction and promote target gene activation. Although we find that histone H4 is inducibly acetylated over a cAMP-responsive promoter, our studies do not exclude the acetylation of other proteins, such as $hTAF_{II}130$, that may potentiate transcription following this modification.

The requirement of CBP HAT activity to initiate transcription via CREB suggests the potential involvement of histone deacetylases (HDACs) in the attenuation process. Indeed, HDAC inhibitors have been found to potentiate CREB activity, in part by prolonging CREB phosphorylation in response to cAMP stimuli (23). Should nucleosome acetylation over the promoter be critical for transcriptional induction via CREB as our results suggest, then deacetylation of promoter-bound nucleosomes would be expected to parallel the loss of transcriptional activity during the attenuation phase.

In addition to its HAT activity, p300 was found to promote transcription via the C/H3 domain. In this regard, p300 has been shown to recruit pol II complexes via its C/H3 domain (25); and recruitment of CBP-pol II complexes to the promoter has been detected by chromatin immunoprecipitation assays of the IFN enhanceosome (1) as well as an estrogen-

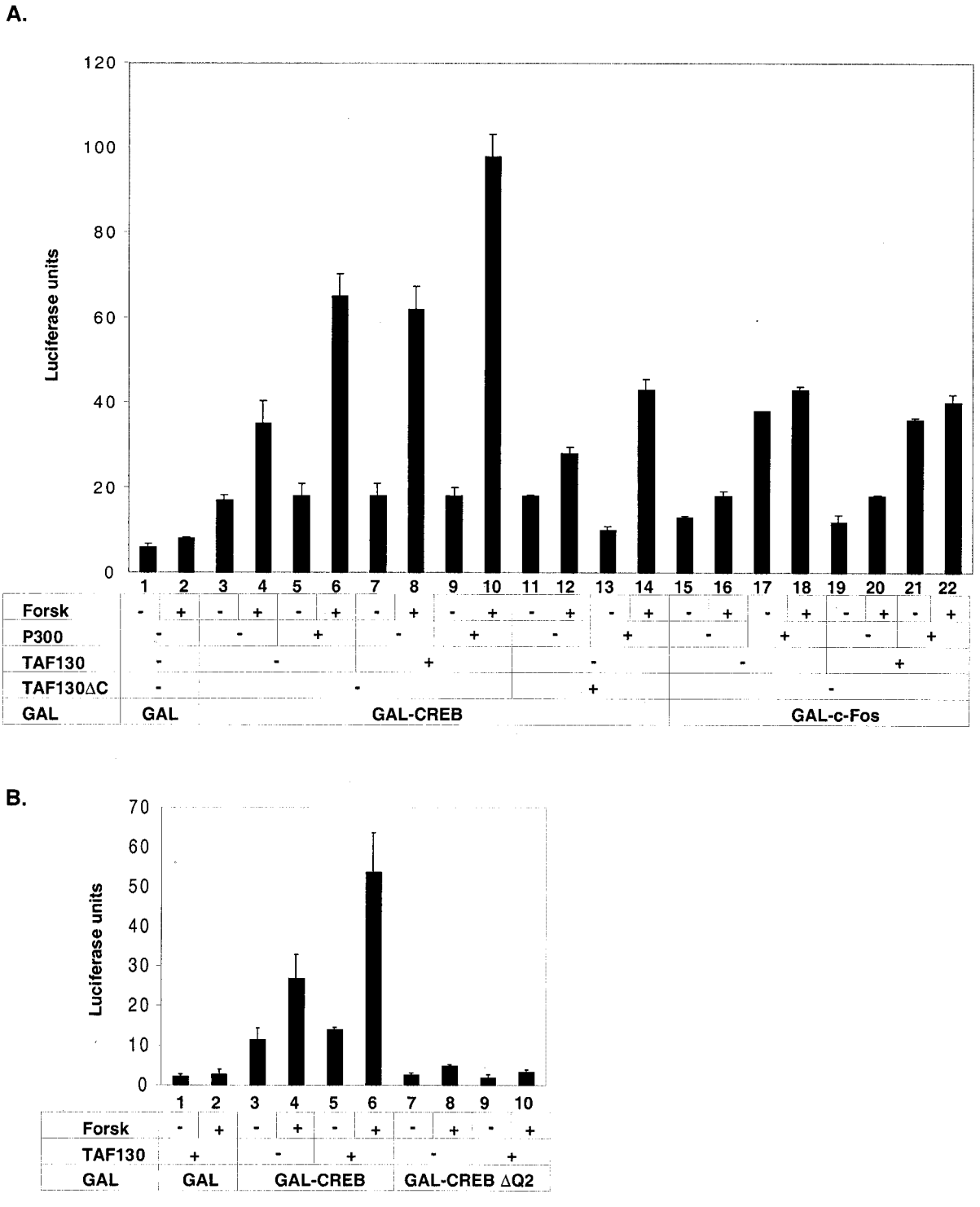


FIG. 6. CRII and Q2 domains in $TAF_{II}130$ and CREB, respectively, are required for $TAF_{II}130$ to stimulate target gene expression in response to cAMP. (A) Transient transfection assay of KD-1 cells containing integrated copies of a GAL4 luciferase reporter gene. Cells were transfected with GAL4-CREB, GAL4-c-fos, or GAL4 DNA binding domain expression vectors as indicated. Cotransfection with p300 as well as wild-type and CREB interaction-defective mutant $TAF_{II}130$ ($TAF_{II}130\Delta C$) constructs is shown. Treatment with cAMP agonist (Forskolin, 10 μM, 4 h) is also indicated. (B) The glutamine-rich Q2 domain in CREB is required for target gene activation via $TAF_{II}130$. Transient transfection of KD-1 cells with wild-type and mutant GAL4 CREB (GAL4-CREBΔQ2) expression vector lacking the Q2 domain, which associates with $TAF_{II}130$, is shown. Cotransfection with $TAF_{II}130$ expression vector and treatment with forskolin is indicated. The average ± standard error for triplicate samples is shown.

regulated promoter (36). Consistent with results reported here, the time course of CBP recruitment coincides with po1 II and actually precedes TFIID assembly over the promoter (1). By acetylating promoter-bound nucleosomes, CBP and p300 may promote subsequent recruitment of TFIID on the promoter via activation domains such as Q2. These results may explain why, like the yeast coactivator GCN5 (19), recruitment of p300 HAT activity to the promoter via the KID domain is necessary but not sufficient for transcriptional induction. Future studies with purified chromatin assembly and general transcription factor components should allow for more precise characterization of this regulatory mechanism.

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