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The CBP co-activator stimulates E2F1/DP1 activity

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ABSTRACT

The cell cycle-regulating transcription factors E2F1/DP1 activate genes whose products are required for S phase progression. During most of the G_1 phase, E2F1/DP1 activity is repressed by the retinoblastoma gene product RB, which directly contacts the E2F1 activation domain and silences it. The E2F1 activation domain has sequence similarity to the N-terminal activation domain of $E1A_{12S}$, which contains binding sites for CBP as well as RB. Here, we present evidence that the CBP protein directly contacts E2F1/DP1 and stimulates its activation capacity. We show that CBP interacts with the activation domain of E2F1 both *in vitro* and *in vivo*. Deletion of four residues from the E2F1 activation domain reduces CBP binding as well as transcriptional activation, but still allows the binding of RB and MDM2. This deletion removes residues which are conserved in the N-terminal activation domain of E1A and which are required for the binding of CBP to E1A. When the E1A N-terminus is used as a competitor in squelshing experiments it abolishes CBP–induced activation of E2F1/DP1, whereas an E1A mutant lacking CBP binding ability fails to do so. These results indicate that CBP can act as a coactivator for E2F1 and suggest that CBP recognises a similar motif within the E1A and E2F1 activation domains. The convergence of the RB and CBP pathways on the regulation of E2F1 activity may explain the cooperativity displayed by these proteins in mediating the biological functions of E1A. We propose a model in which E1A activates E2F not only by removing the RB repression but also by providing the CBP co-activator.

INTRODUCTION

The E2F1 transcription factor is a member of the E2F family (1,2), which is composed of five E2F proteins and three DP proteins (reviewed in 3). E2F1 binds DNA as a heterodimer with the DP1 protein and activates transcription through a C-terminal activation domain (2,4,5,16).

Many of the E2F target genes contribute to DNA synthesis. E2F binding sites have been identified in the promoters of the c-*myc*, c-*myb*, N-*myc*, dihydrofolate reductase (DHFR), thymidine kinase, DNA polymerase α and cdc2 genes (reviewed in 7,8). In some of these cases, the E2F site has been shown to be involved in serum response and appropriate expression at the G_1–S boundary (9,10). Futhermore, deregulated expression of E2F1 is able by itself to induce cell entry into S phase (11) and can cause transformation in cooperation with an activated ras oncogene (12,13). These observations suggest that E2F1 plays a major role in S phase induction during the normal cell cycle.

E2F1 activity is regulated at the protein level during the G_1 phase of the cell cycle by the product of the retinoblastoma susceptibility gene RB. The hypophosphorylated form of RB contacts directly the E2F1 activation domain and represses its activity (1,2,14,15). The ability of RB mutants to repress E2F1 correlates with their capacity to suppress cell growth (16). Futhermore, expression of E2F1 overcomes the growth inhibitory effects of RB (17,18), indicating that the suppression of E2F1 activity is likely to be involved in growth suppression by RB.

The E2F1 activation domain consists of ~60 amino acids located at the very C-terminus of the protein (2,4,15). This activation domain has been shown to interact directly with TBP (TATA binding protein) *in vitro* using residues also involved in transcriptional activation, suggesting that direct binding to TBP may participate in E2F1 transactivation properties (4). However, some mutants of E2F1 unable to bind TBP can still activate transcription, albeit at a lower level, indicating that alternate mechanisms exist (4). A likely possibility is that proteins functioning as co-activators are involved in mediating the activity of the E2F1 activation domain. One such protein is the product of the mdm2 oncogene, MDM2, which possesses many of the characteristics of an E2F1 co-activator (19). MDM2 binds directly to the E2F1 activation domain and stimulates its activation function. The mechanism of MDM2-mediated stimulation is unclear. Displacement of RB from the E2F1 activation domain is unlikely to be the mechanism, considerating that the binding sites for MDM2 and RB are not overlapping.

One characterized co-activator is the adaptor protein CBP (CREB binding protein), involved in transactivation by phosphorylated CREB (20,21). CBP contacts the phosphorylated form of CREB and activates transcription through a C-terminal activation domain (21,22). The CBP adaptor protein is also implicated in transcriptional activation by the Fos/Jun complex (23,24).

CBP is highly homologous to the E1A-associated protein p300 (25,26), and is itself a target of E1A (27,28). The functions of CBP and p300 have so far been indistinguishible. Both proteins

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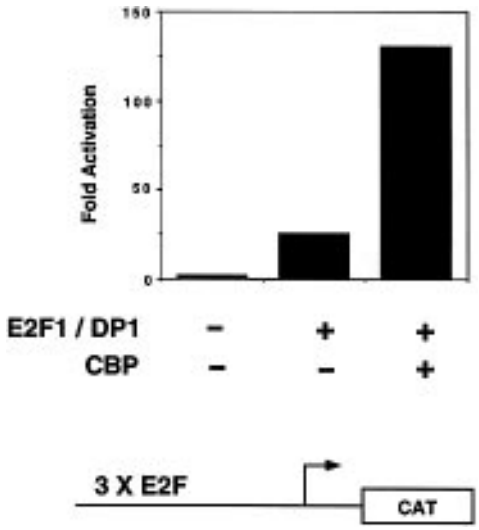

Figure 1. CBP activates E2F1/DP1 activity. U2OS cells were transiently transfected with 2 µg of (E2F)3TKCAT reporter vector and where indicated 100 ng of CMV E2F1, 100 ng of CMV DP1 and 4 µg of RSV CBP. The amount of promoter was kept constant by the addition when necessary of CMV and RSV empty vectors. Following a CAT assay, results were quantified using a phosphorimager. The activity of the reporter in the absence of E2F1, DP1 and CBP is normalised to a value of 1.

act as coactivators of CREB-induced transcriptional activation and E1A can interact with both CBP and p300 using similar sequences within E1A CR1.

In this paper, we show that CBP augments E2F1/DP1 activity. Using *in vitro* and *in vivo* assays, we demonstrate that the target of this activation is the E2F1 activation domain. Residues required for the binding of CBP to E2F1 are conserved in E1A and are required for CBP interaction. These results suggest that CBP can function as a co-activator for E2F1 and that it can recognise the E2F1 and E1A activation domains via similar sequences.

RESULTS

We have recognised a sequence similarity between the activation domain of E2F1 and the N-terminus of E1A (29). The similarity includes residues in E1A which are required for binding to the p300/CBP family of co-activators. This prompted us to examine whether the activation functions of the E2F1/DP1 heterodimer are stimulated by the CBP adaptor protein. Figure 1 shows the E2F1 and DP1 proteins activating the expression of a CAT gene linked to E2F-binding sites; when the CBP protein is co-expressed in these cells, the activation capacity of E2F1/DP1 is stimulated 5-fold. This stimulation is comparable with that observed when CREB is the target for CBP (21).

The N-terminal region of E1A (1–90; E1A-N) has a high affinity binding site for CBP (30) and shows sequence similarity to the E2F1 activation domain (Fig. 2A). To confirm that the stimulation of E2F1/DP1 was the direct result of CBP activity we asked whether E1A-N can compete for this activity. To make sure that E1A-N is sufficient for CBP binding, we first tested its ability to 'squelch' the activity of the SV40 promoter–enhancer, since squelching of this promoter by E1A has been shown to be due to the binding of the CBP related protein p300 (26). Figure 2B shows that E1A-N can repress the activity of pSV2-CAT. This repression is not seen when an E1A mutant is used (E1A-N p300mut; Fig. 2A) which cannot bind p300 *in vivo* (31). In contrast, an E1A mutant which cannot bind RB but can bind p300 (E1A-N RB mut; Fig. 2A) (31) can still repress SV40 promoter activity. The repressive effect of E1A-N or E1A-N RBmut is aleviated when CBP is co-expressed (Fig. 2C). These results confirm that CBP, like p300 (26), can relieve SV40 enhancer repression by E1A and that E1A-N is sufficient for CBP binding.

Next we used E1A-N as a competitor for E2F1/DP1 activity. Figure 2D shows that E1A-N can repress the CBP stimulated activity of E2F1/DP1. The repression is even more marked when a mutant of E1A-N (E1A-N RBmut) is used, which abolishes binding of RB to E1A (31). This mutant is therefore a better 'squelching' agent because it does not sequester RB from E2F1/DP1, which would result in increased activity. The squelching ability of E1A-N correlates with the ability to bind p300 since a mutant that cannot bind CBP/p300 (E1A-N p300mut) cannot compete for this activity (Fig. 2D). This mutant shows a slight stimulation of activity consistent with the fact that it is still capable of binding the RB protein.

The CBP protein is considered to be a co-activator protein which functions to bridge the activation domain of CREB to the basal machinery. Therefore, we considered the possibility that CBP may be functioning as a co-activator for E2F1. We first sought to establish whether CBP directly contacts E2F1.

The CBP protein has two characterised binding sites for transcription factors. One of these encompasses residues 461–662 (CBP1 domain; Fig. 3) and represents the binding site for CREB (22). The other encompasses residues 1621–1877 (CBP 2 domain; Fig. 3) and represents the binding site for E1A and c-Fos. Each of these domains (CBP 1 and CBP 2) was expressed in bacteria, radiolabelled *in vitro* and added to GST–E2F1 and GST–DP1 in a GST–pull down assay. Figure 3 shows that E2F1 and DP1 bound specifically to the CBP 2 domain but not to CBP 1. The specificity of E2F1 and DP1 for the CBP 2 domain is further supported by our previous observations that, under similar conditions, the CREB transcription factor, when phosphorylated by protein kinase A, binds to the CBP 1 domain and not CBP 2 (23). These results indicate that E2F1 and DP1 bind directly to the domain of CBP which also binds E1A and that, unlike the CREB transcription factor, these two proteins do not require prior phosphorylation to contact CBP.

The E2F1 activation domain has sequence similarity to the N-terminal region of E1A which can bind CBP (Fig. 2A). Therefore, we tested whether the activation domain of E2F1 was sufficient for the interaction with CBP. Figure 4 shows that the E2F1 activation domain (GST–E2F-C) can independently bind the CBP 2 domain.

Dissection of the E2F1 activation domain has shown that it is composed of at least three activation modules (Fig. 4). Each module is unable to activate transcription efficiently when linked to heterologous DNA binding domain (GAL4) but can do so very efficiently in combination with a second E2F1 activation module (4). Removal of module 3 sequences (426–437) from the E2F1 activation domain abolishes the interaction between CBP and E2F1 but still allows binding to MDM2 and RB (Fig. 4). Within the sequence of E2F1 module 3, there is sequence similarity with E1A residues required for the binding of p300/CBP (Fig. 2). Mutagenesis of these residues in E1A abolishes CBP/p300 binding (31) and prevents 'squelshing' of SV40 enhancer and E2F1/DP1 activity (Fig. 2). To establish whether the E1A-related

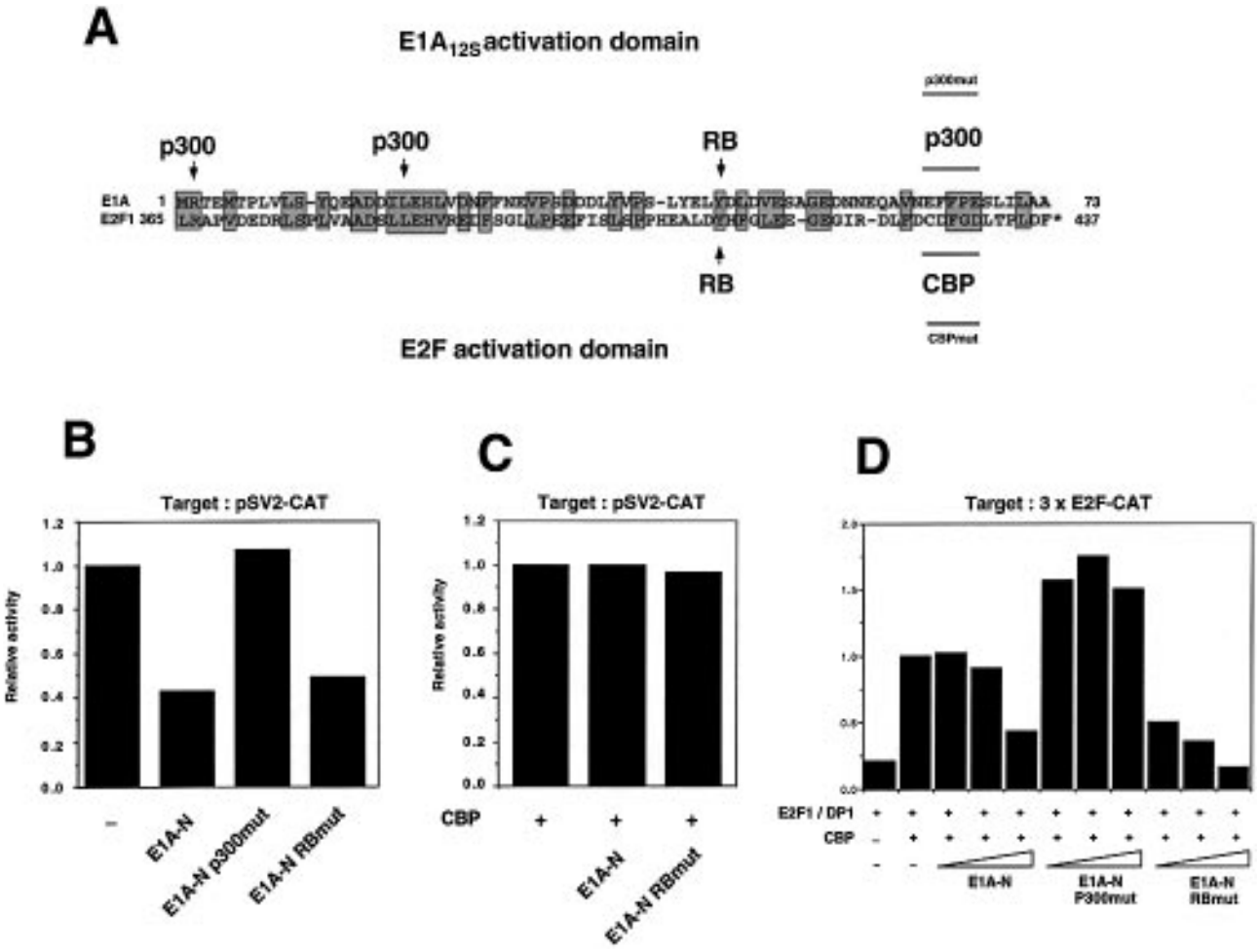

Figure 2. The E1A N-terminus sequesters CBP activity. (**A**) Alignment of the Ad12 E1A N-terminus (amino acids 1–73) with the E2F1 activation domain (amino acids 365–437) (29). Residues implicated in the binding of RB and p300 in E1A and E2F are indicated. Mutants generated to address CBP/p300 binding are indicated (p300mut, CBPmut). (**B**) U2OS cells were transfected with 1 µg of SV40 enhancer containing pSV2-CAT reporter vector together with 2 µg of the indicated E1A-N expressor construct. E1A-N p300mut and E1A-N RBmut are no longer able to bind p300/CBP or RB respectively. The amount of promoter was kept constant by the addition when necessary of pHK empty vector. The activity of the reporter in the absence of E1A-N expression vector is normalised to a value of 1. (**C**) Same as in (A), in the presence of 4 µg of RSV-CBP expression vector. (**D**) The E1A N-terminus represses CBP–activated E2F activity. U2OS cells were transfected with 2 µg (E2F)3TKCAT reporter vector, 100 ng CMV E2F1, 100 ng CMV DP1. Where indicated, 4µg RSV CBP and 5, 10 or 15 µg of the indicated pHK E1A-N expression vector were also included. The amount of promoter was kept constant by the addition when necessary of RSV and pHK empty vectors. The activity of the reporter in the presence of CBP and in the absence of E1A-N expression vector is normalised to a value of 1.

residues in E2F1 module 3 were also involved in CBP binding, we deleted them from the E2F1 activation domain. This four residue deletion (E2F-C CBPmut) abolishes binding of E2F1 to CBP (Fig. 4) but does not affect binding to MDM2 or RB. This result strongly supports the alignment of the $E1A_{12S}$ and E2F1 shown in Figure 2A, and suggests that CBP recognises similar residues in E1A and E2F1.

Deletion of module 3 (E2F1 Δm3) severely affects the activation capacity of E2F1 (ref. 4 and Fig. 4). The same is true for the E2F-C CBPmut: when this mutant is linked to GAL4, it activates transcription at levels comparable with E2F1 Δm3 (ref. 29 and Fig. 4). These results indicate that E2F1 residues required for the binding of CBP are also required for transcriptional activation. This is compatible with a role for CBP as a coactivator of E2F1 activity.

These data, presented in Figures 3 and 4, point to a direct interaction between the CBP2 domain of CBP and the activation domain of E2F1 *in vitro*. We next sought to verify that these domains can also interact *in vivo*. To do this we used the 'two hybrid' assay in U2OS cells (Fig. 5). For this purpose, the CBP 1 or CBP 2 domain was fused to the VP16 activation domain. We then asked whether either of these chimeras could augment the activation capacity of a GAL4 E2F1-C fusion. Figure 5A shows that the activity of the E2F1 activation domain (GAL4 E2F1-C) is specifically stimulated by CBP2–VP16 but not CBP1–VP16 or VP16 alone. In contrast, neither CBP1 nor CBP2–VP16 will augment the activation capacity of the SP1 activation domain (GAL4 SP1-AD) (Fig. 5B). Consistent with a co-activator activity for E2F1, the CBP2–VP16 fusion will also augment activation of a promoter driven by E2F sites (Fig. 5C). Further evidence of the specificity of this two hybrid assay comes from experiments using the CREB activation domain as a target. We can show that under similar conditions, it is the CBP1–VP16 fusion (and not CBP2–VP16) which is capable of stimulating

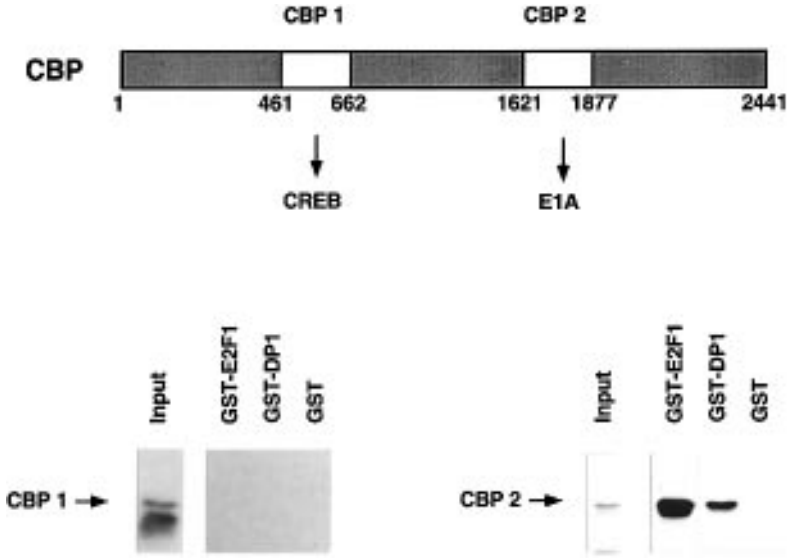

Figure 3. E2F1 interacts with CBP domain 2 *in vitro*. ^{32}P-labelled bacterially expressed CBP domain 1 (CBP1, left) or CBP domain 2 (CBP2, right) were subjected to a pull down using beads coated with the indicated GST fusion protein. After extensive washing, bound proteins were resolved by SDS–PAGE analysis. Gels were dried and autoradiographed. Upper part: map of the CBP protein indicating the previously characterized interaction domains with CREB (domain 1) or with E1A (domain 2). These domains were used in the pull-down assay.

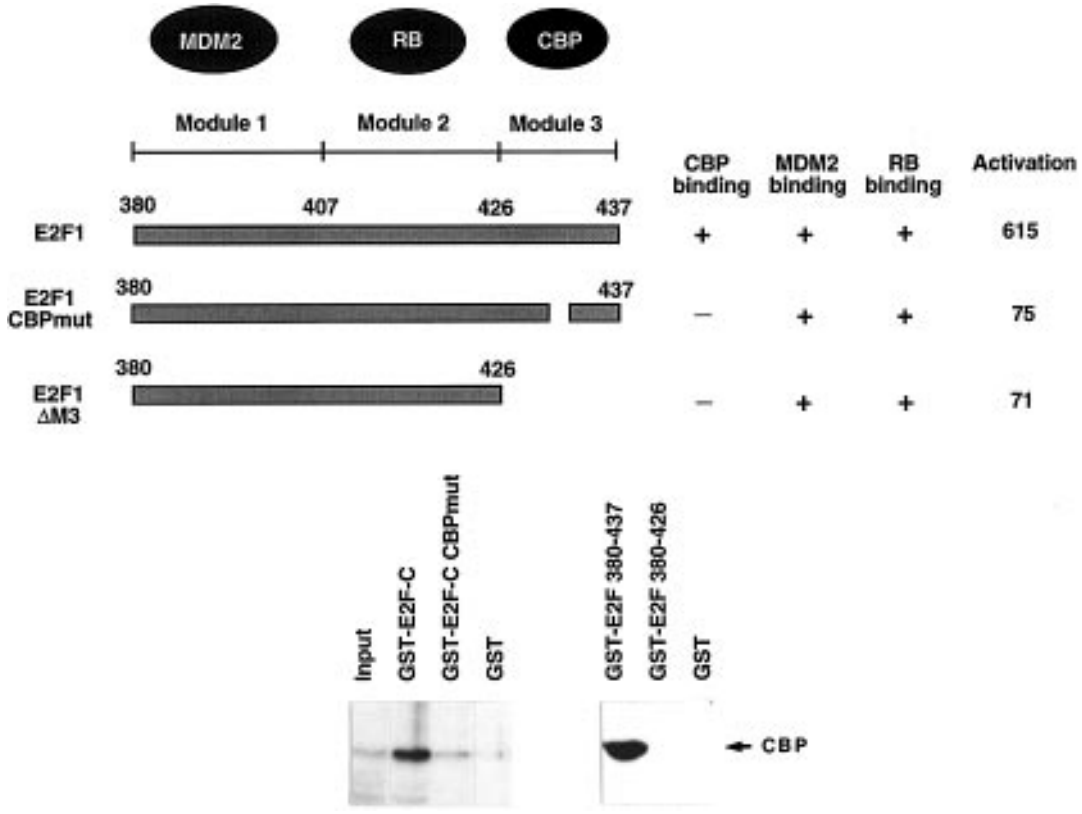

Figure 4. CBP interacts with E2F1 activation domain *in vitro*. ^{32}P-labelled bacterially expressed CBP domain 2 (CBP2) was subjected to an *in vitro* pull-down using beads coated with the indicated part of E2F1 activation domain fused to GST or with control GST alone. E2F-C and E2F-CΔM3 represent E2F1 residues 380–437 and 380–426 respectively. After extensive washing, bound proteins were resolved by SDS–PAGE analysis. The upper part showed the position of the CBP binding site on E2F1 activation domain together with the previously defined binding sites for MDM2 (19) and RB (4). Protein binding and transcriptional activation capacities (4,29) of some E2F1 activation domain mutants are also indicated on the right. Note the correlation between CBP binding and transcriptional activation.

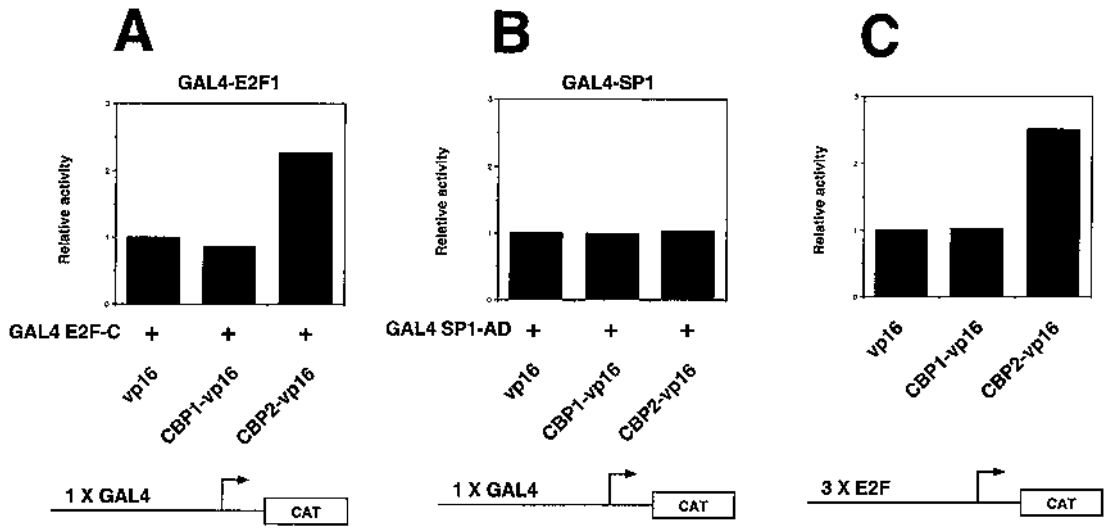

Figure 5. *In vivo* two-hybrid interaction between E2F1 and CBP. (**A**) U2OS cells were transfected with 1 µg 1GE1bCAT and 1 µg pHKG E2F-C. Also transfected was 1 µg of either pHK VP16 (expressing VP16AD), pHK CBP1–VP16 (expressing CBP1–VP16AD) or pHK CBP2–VP16 (expressing CBP2–VP16AD) when indicated. The activity of the effector in the presence of VP16AD alone is normalised to a value of 1. (**B**) Same as in (A) with 1 µg pHKG Sp1-AD. (**C**) U2OS cells were transfected with 1 µg (E2F)3TK CAT and when indicated pHK VP16 (expressing VP16AD), pHK CBP1–VP16 (expressing CBP1–VP16AD) or pHK CBP2–VP16 (expressing CBP2–VP16AD). The activity of the reporter in the presence of VP16AD alone is normalised to a value of 1.

GAL4 CREB-AD activity, consistent with the fact that CBP1 (and not CBP2) is the domain that interacts with CREB *in vitro* (23).

The level of stimulation by CBP–VP16 in Figure 5 and reference 23 is only a few fold, suggesting that other parts of CBP may stabilize the interaction. However, the fold stimulation is comparable with that is seen by CBP, is highly reproducible, and precisely reflects specific interactions that occur *in vitro*. These experiments provide evidence that the CBP 2 domain of the CBP protein is able to bind E2F1 activation domain *in vivo*. We have not been successful in showing a direct association between CBP and E2F1 or DP1 using co-immunoprecipitation assays. This may reflect the low affinity of the interaction *in vivo*, compared with E1A, or a limitation in the currently available CBP antibodies. Consistent with this interpretation is the fact that co-immunoprecipitations have not been reported for the interaction between CBP and CREB.

DISCUSSION

The CBP protein is considered to be a coactivator protein capable of mediating the activation functions of the CREB transcription factor. The results presented here provide evidence that CBP also has characteristics expected of a co-activator for E2F1-mediated transcription: (i) CBP stimulates the activity of the E2F1/DP1 heterodimer; (ii) CBP interacts directly with the E2F1 activation domain; (iii) CBP contacts E2F1 residues required for transcriptional activation; and (iv) CBP recognises E2F1 residues which show sequence similarity to the CBP–binding residues in E1A.

Characterization of the E2F1 activation domain can help in understanding the mechanism by which RB suppresses its activity. There are now three proteins, MDM2, CBP and TBP which can directly contact this domain and have the capacity to mediate its activation functions. Each of these proteins can contact distinct, cooperative activation modules within the E2F1 activation domain. The cooperativity between these modules can be explained by their ability to bind these distinct proteins which may be involved in regulating different steps in transcription.

One obvious mechanism by which RB may suppress E2F1 activity is that it may compete for the binding of one or more of these proteins. Consistent with this hypothesis is the fact that the binding site for RB and TBP is indistinguishible by deletion and point mutational analysis, suggesting that the binding of these two proteins is mutually exclusive (4). This raises the possibility that RB can silence the activation domain of E2F1 by displacing TBP (TFIID) from the E2F1 activation domain. This may be a common mechanism for RB induced repression. The RB protein can also contact the activation domain of Pu1 using residues which overlap the binding site for TBP (32). RB can repress the activation capacity of Pu1, after RB is recruted to the promoter, and this repression correlates with the displacement of TBP and TFIID from the Pu1 protein (33).

In contrast with TBP, the MDM2 protein recognises sequences within the E2F1 activation domain that are distinct from those that bind RB (19). RB is unlikely, therefore, to displace MDM2 from E2F1. However, since RB can form a complex with MDM2 (34), it may mask the activation capacity of MDM2 by direct contact.

The binding of the CBP protein to the E2F1 activation domain indicates that CBP contacts residues distinct from those required to bind RB (Fig. 4). However, module 3, which is required for CBP binding, is not sufficient for CBP interaction, suggesting that additional residues within modules 1 and 2 are required for CBP contact (unpublished results). This is consistent with the sequence similarity of this region to E1A, since CBP/p300 can recognise a number of different residues along the E1A activation domain (Fig. 2). Since these residues fall N- and C-terminal to the RB binding site, this leaves open the possibility that the binding of CBP and RB to the E2F1 activation domain may be mutually exclusive due to steric hindrance.

This brings us to the question of how the binding of CBP and RB to E1A relates to the abilitiy of E1A to stimulate the activation capacity of E2F. Although the data presented in Figure 2 show

that the N-terminus of E1A can squelch E2F activity, this is only true when the N-terminus is released from the rest of the E1A protein and is not seen with full length E1A. Instead, $E1A_{12S}$ stimulates E2F activity. Mutating the CBP binding site within the $E1A_{12S}$ background reduces E1A-mediated stimulation, indicating that the E1A–CBP interaction is required for the ability of E1A to stimulate E2F (29). We favor the model whereby E1A acts as a carrier of the CBP coactivator, providing it to E2F1, while at the same time, binding and removing the RB repressor.

This model is perfectly consistent with recent results showing that E1A is able to bind RB and p300 simultaneously. To do this, E1A uses the LXCXE motif in Conserved Region 2 (CR2) to bind RB and the N-terminal CR1 domain to bind CBP (35). Furthermore, this trimeric complex seems essential for the biological properties of E1A, since both RB and p300 have to be bound to the same E1A protein in order to induce cell proliferation (35).

These data argue against E1A simply squelching away CBP and RB but support the argument that the biological properties of CBP and RB are closely linked. Thus, E1A has to bring these two proteins together to accomplish its functions. Given the data presented here, that CBP as well as RB are involved in regulating the activity of E2F, it is tempting to speculate that regulation of E2F1 is the molecular link responsible for the functional cooperation of RB and CBP in mediating the biological effcts of E1A.

MATERIALS AND METHODS

Cell culture, transfections and CAT assays

U2OS human osteosarcoma cells were maintained in DMEM supplemented with 10% fetal calf serum and grown at 37°C (5% CO_2). Subconfluent cells were transfected overnight using the calcium phosphate co-precipitation technique. After 24 h incubation, extracts from transfected cells were used for CAT assays. CAT assays were quantitated by a PhosphorImager.

In vivo expression plasmids

(E2F)3TKCAT, CMV-E2F1 and CMV-DP-1 have been described previously (19). CBP was expressed under the control of an RSV promoter and was a gift from R. Goodman. 1GE1bCAT and pHKG Sp1-AD expression plasmid are kind gifts from M. Green and S. P. Jackson respectively. pHKG E2F-C expression vector contains E2F1 sequences from amino acids 380–437 in frame with GAL4 DNA binding domain and has been described previously (4). Plasmid pHK VP16 expressed the VP16 activation domain under the control of the SV40 promoter. Plasmids pHK CBP1–VP16 and pHK CBP2–VP16 expressed CBP 461–662 and CBP 1621–1877 respectively in frame with the VP16 activation domain (24). pHK E1A-N expressed Ad5 E1A sequences from 1 to 90 in frame with the large T antigen Nuclear Localisation Signal under the control of the SV40 promoter. Plasmid pHK E1A-N p300mut contained a deletion of amino acids 64–68. In plasmid pHK E1A-N RBmut, amino acids 38–44 from E1A were converted to alanine.

GST fusion proteins and pull-down assay

CBP domain 1 (461–662) and domain 2 (1621–1877) were cloned into the relevant pGex vector (Pharmacia), using PCR or engineered restriction sites. Plasmids expressing GST–E2F1, GST–DP1, GST E2F1-C (containing E2F1 sequences from 380 to 437) were described previously (19). Plasmid expressing GST–E2F1-C ΔM3 has been constructed by inserting E2F1 sequences from 380 to 426 into pGEX-2TK. To create plasmids expressing GST–E2F 359–437 and GST–E2F 359–437 CBPmut, E2F1 coding sequences from 359 to 437 were excised from GAL-E2F1 359–437 (4) and mutant respectively and cloned in frame with GST in pGEX-2TK. Recombinant proteins were expressed in and purified from *Escherichia coli* as reported previously (36). Pull-down assays were performed as described previously (32). Briefly, 1 µg of fusion protein on beads was incubated 10 min at 20°C in 200 µl Z′ buffer (25 mM HEPES pH 7.5, 12.5 mM $MgCl_2$, 20% glycerol, 0.1% NP-40, 150 mM KCl, 1 mM DTT) with 100 µg BSA. In some experiments, 5 µl of reticulocyte lysate were included to increase specificity. Labelled protein was then added and incubation was allowed to continue for 1 h at 20°C. Beads were collected and washed three times in NETN (20 mM Tris pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5% NP-40). Beads were then boiled in 1× SDS sample buffer, and bound proteins analysed by SDS–PAGE.

^{32}P-labelling of GST fusion proteins

Proteins to be ^{32}P-labelled were cloned into pGEX-2TK (kindly provided by W. Kaelin). This plasmid expresses a fusion protein which contains a protein kinase A (PKA) phosphorylation site directly adjacent to the C-terminus of the thrombin cleavage site. The proteins were labelled according to ref. 2. Briefly, ~1 µg of fusion protein on beads was labelled in 30 µl HMK buffer (20 mM Tris pH 7.5, 100 mM NaCl, 12 mM $MgCl_2$, 1 mM DTT) with 3 µl of [χ-^{32}P]ATP (NEN) and 10 U Protein Kinase A (catalytic subunit, Sigma). After 30 min on ice, beads were washed extensively with 1× TBS (20 mM Tris pH 8, 100 mM NaCl) then labelled protein was cut 2 h at 26°C with 1 µg thrombin (Sigma) in TBS buffer supplemented with 1 mM $CaCl_2$ and 0.5 urea. Following addition of EGTA, the supernatant was used in pull-down assays.

REFERENCES

1 Helin, K., Lees, J. A., Vidal, M., Dyson, N., Harlow, E. and Fattaey, A. (1992) *Cell*, **70**, 337–350.
2 Kaelin, W. K., Jr, Krek, W., Sellers, W. R., DeCaprio, J.A., Ajchenbaum, F., Fuchs, C. S., Chittenden, T., Li, Y., Farnham, P. J., Blanar, M. A., Livingston, D. M. and Flemington, E. K. (1992) *Cell*, **70**, 351–364.
3 Lam, E. W.-F. and La Thangue, N. B. (1994) *Curr. Opin. Cell Biol.*, **6**, 859–866.
4 Hagemeier, C., Cook, A. and Kouzarides, T. (1993) *Nucleic Acids Res.*, **21**, 4998–5004.
5 Helin, K., Wu, C. L., Fattaey, A. R., Lees, J. A., Dynlacht, B. D., Ngwu, C. and Harlow, E. (1993) *Genes Dev.*, **7**, 1850–1861.
6 Wu, C. L., Zukerberg, L. R., Ngwu, C., Harlow, E. and Lees, J. A. (1995) *Mol. Cell. Biol.*, **15**, 2536–2546.
7 Nevins, J. R. (1992) *Science*, **258**, 424–429.
8 La Thangue, N. B. (1994) *Trends Biochem. Sci.*, **19**, 108–114.
9 Mudryj, M., Hiebert, S. W. and Nevins, J. R. (1990) *EMBO J.*, **9**, 2179–2184.
10 Means, A. L., Slansky, J. E., McMahon, S. L., Knuth, M. W. and Farnham, P. J. (1992) *Mol. Cell. Biol.*, **12**, 1054–1063.
11 Johnson, D. G., Schwarz, J. K., Cress, W. D. and Nevins, J. R. (1993) *Nature*, **365**, 349–352.
12 Johnson, D. G., Cress, W. D., Jakoi, L. and Nevins, J. R. (1994) *Proc. Natl Acad. Sci. USA*, **91**, 12823–12827.
13 Singh, P., Heng Wong, S. and Hong, W. (1994) *EMBO J.* **13**, 3329–3338.
14 Helin, K., Harlow, E. and Fattaey, A. (1993) *Mol. Cell. Biol.*, **13**, 6501–6508.

15 Flemington, E. K., Speck, S. H. and Kaelin, W. G., Jr (1993) *Proc. Natl. Acad. Sci. USA*, **90**, 6914–6918.
16 Qin, X.-Q., Chittenden, T., Livingston, D. M. and Kaelin, W. G., Jr (1992) *Genes Dev.*, **6**, 953–964.
17 Zhu, L., van den Heuvel, S., Helin, K., Fattaey, A., Ewen, M., Livingston, D. M., Dyson, N. and Harlow, E. (1993) *Genes Dev.*, **7**, 1111–1125.
18 Qin, X. Q., Livingston, D. M., Ewen, M., Sellers, W. R., Arany, T. and Kaelin, W. G., Jr (1995) *Mol. Cell. Biol.*, **15**, 742–755.
19 Martin, K., Trouche, D., Hagemeier, C., Sorensen, T., La Thangue, N. B. and Kouzarides, T. (1995) *Nature*, **375**, 691–694.
20 Arias, J., Alberts, A. S., Brindle, P. Claret, F. X., Smeal, T., Karin, M., Feramisco, J. and Montminy, M. (1994) *Nature*, **370**, 226–229.
21 Kwok, R. P. S., Lundblad, J. R., Chrivia, J. C., Richards, J. P., Bächinger, H. P., Brennan, R. G., Roberts, S. G. E., Green, M. R. and Goodman, R. H. (1994) *Nature*, **370**, 223–226.
22 Chrivia, J. C., Kwok, R. P. S., Lamb, N., Hagiwara, M., Montminy, M. R. and Goodman, R. H. (1993) *Nature*, **365**, 855–859.
23 Bannister, A. J. and Kouzarides, T. (1995) *EMBO J.*, **14**, 4758–4762.
24 Bannister, A. J., Oehler, T., Wilhelm, D., Angel, P. and Kouzarides, T. (1995) *Oncogene*, **11**, 2509–2514.
25 Arany, Z., Sellers, W. R., Livingston, D. M. and Eckner, R. (1994) *Cell*, **77**, 799–800.
26 Eckner, R., Ewen, M. E., Newsome, D., Gerdes, M., DeCaprio, J. A., Bentley- Lawrence, J. and Livingston, D. M. (1994) *Genes Dev.*, **8**, 869–884.
27 Arany, Z., Newsome, D., Oldread, E., Livingston, D. M. and Eckner, R. (1995) *Nature*, **374**, 81–84.
28 Lundblad, J. R., Kwok, R. P. S., Laurance, M. E., Harter, M. L. and Goodman, R. H. (1995) *Nature*, **374**, 85–88.
29 Trouche, D. and Kouzarides, T. (1996) *Proc. Natl. Acad. Sci. USA*, **93**, 1439–1442.
30 Wang, H.-G. H., Rikitake, Y., Carter, M. C., Yaciuk, P., Abraham, S. E., Zerler, B. and Moran, E. (1993) *J. Virol.*, **67**, 476–488.
31 Wong, H. K. and Ziff, E. B. (1994) *J. Virol.*, **68**, 4910–4920.
32 Hagemeier, C., Bannister, A. J., Cook, A. and Kouzarides, T. (1993) *Proc. Natl. Acad. Sci. USA*, **90**, 1580–1584.
33 Weintraub, S. J., Chow, K. N. B., Luo, R. X., Zhang, S. H., He, S. and Dean, D. C. (1995) *Nature*, **375**, 812–815.
34 Xiao, Z.-X., Chen, J., Levine, A. J., Modjtahedi, N., Xing, J., Sellers, W. R. and Livingston, D. M. (1995) *Nature*, **375**, 694–697.
35 Wang, H.-G. H., Moran, E. and Yaciuk, P. (1995) *J. Virol.*, **69**, 7917–7924.
36 Bannister, A. J., Cook, A. and Kouzarides, T. (1991) *Oncogene*, **6**, 1243–1250.