E1A represses apolipoprotein AI enhancer activity in liver cells through a pRb- and CBP-independent pathway

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ABSTRACT

The apolipoprotein AI (apoAI) promoter/enhancer contains multiple *cis*-acting elements on which a variety of hepatocyte-enriched and ubiquitous transcription factors function synergistically to regulate liver-specific transcription. Adenovirus E1A proteins repress tissue-specific gene expression and disrupt the differentiated state in a variety of cell types. In this study expression of E1A 12S or 13S in hepatoblastoma HepG2 cells repressed apoAI enhancer activity 8-fold. Deletion mapping analysis showed that inhibition by E1A was mediated by the apoAI promoter site B. E1A selectively inhibited the ability of HNF3β and HNF3α to transactivate reporter genes controlled by the apoAI site B and the HNF3 binding site from the transthyretin promoter. The E1A-mediated repression of HNF3 activity was not reversed by overexpression of HNF3β nor did E1A alter nuclear HNF3β protein levels or inhibit HNF3 binding to DNA in mobility shift assays. Overexpression of two cofactors known to interact with E1A, pRb and CBP failed to overcome inhibition of HNF3 activity. Similarly, mutations in E1A that disrupt its interaction with pRb or CBP did not compromise its ability to repress HNF3β transcriptional activity. These data suggest that E1A inhibits HNF3 activity by inactivating a limiting cofactor(s) distinct from pRb or CBP.

INTRODUCTION

Hepatocyte-specific expression is maintained primarily by four families of liver-enriched transcription factors: the hepatocyte nuclear factor 3 (HNF3) family, the hepatocyte nuclear factor 1 (HNF1) family, the CCAAT enhancer binding proteins (C/EBP) family and various members of the nuclear receptor superfamily, such as hepatocyte nuclear factor 4 (HNF4) (reviewed in 1). Liver-specific genes are regulated by promoter/enhancer sequences containing closely spaced *cis*-acting elements on which different combinations of hepatocyte-enriched and ubiquitous factors assemble (reviewed in 2).

The gene encoding apolipoprotein AI (apoAI), the predominant protein in the anti-atherosclerotic high density lipoprotein (HDL) (reviewed in 3), contains a powerful liver-specific enhancer

located in the –222/–110 nucleotide region upstream of the apoAI transcription start site (4,5). In hepatocytes, apoAI gene transcription is maintained by three *cis*-acting elements, sites A (–214 to –192), B (–169 to –146) and C (–134 to –119), within the enhancer (5). Sites A and C are bound by members of the nuclear receptor superfamily, including HNF4, ARP-1, RXRα and RAR/RXR heterodimers (6–9). Site B binds the hepatocyte-enriched factors HNF3β, HNF3α and C/EBP (10,11). Recent data suggest that although these factors bind independently to their corresponding sites, they stimulate apoAI enhancer activity synergistically via conjoint recruitment of an uncharacterized transcriptional coactivator(s) (5,11). Two conserved early growth response factor (Egr-1) *cis*-acting elements, designated E1 (–189 to –181) and E2 (–221 to –213), flank site A but do not appear to contribute to apoAI promoter activity under normal conditions (12).

The developmental appearance of HNF3 and HNF4 correlates with endoderm differentiation and liver development (13,14). The apoAI, HNF3α and HNF4 genes are activated upon differentiation of F9 teratocarcinoma cells, an *in vitro* model for endoderm differentiation, into visceral endoderm cells (15–17). The differentiation-dependent expression of these genes is correlated with suppression of an adenoviral E1A-like activity present endogenously in undifferentiated F9 cells (18). E1A also functions as a transcriptional coactivator for RARs and RAR/RXR heterodimers and binds to the integrator protein p300/CBP, which appears to be required for nuclear receptor function (19–24). These observations raised the possibility that E1A-like factors may play a role in regulation of the apoAI enhancer in liver cells.

The adenovirus E1A 12S and 13S gene products are required for viral replication and their expression induces quiescent cells to enter the cell cycle (25,26). Several conserved domains within E1A are required for its effects on cell quiescence (reviewed in 27). 12S E1A differs from 13S E1A by the absence of an alternatively spliced exon (CR3, residues 140–185) that is involved in interactions with various transcription factors such as ATF-2 (28,29). A different domain of E1A (CR2, residues 121–139) interacts with the retinoblastoma tumor suppressor-related proteins, so called 'pocket' proteins, pRb, p130 and p107 (30, reviewed in 27,31,32). The pRb-related proteins associate with and regulate the E2F family of transcription factors that are involved in cell cycle progression (33–36). Interaction of E1A with 'pocket' proteins releases active E2F (30,33,37). The N-terminus of E1A (residues 1–39) interacts with the transcriptional

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coactivators p300/CBP (38–42) and the basic helix–loop–helix (bHLH) domains of the myogenic determination factors myogenin and E12 (43). These interactions play an important role in the interplay between myogenic and cell cycle proteins responsible for maintenance of the differentiated muscle phenotype and cell quiescence (43–46).

In liver the interplay between hepatocyte-enriched factors and ubiquitous regulators of the cell cycle have not been elucidated. In this study E1A was found to be a potent repressor of the apoAI enhancer in liver cells. E1A repressed the apoAI enhancer activity by selective inhibition of the transcriptional activity of HNF3. These findings suggest that E1A-like factors could influence the hepatocyte phenotype by modulating the transcriptional activity of HNF3. The ability of E1A to modulate the activity of a transcription factor such as HNF3 that plays an important role in liver development and gene expression is similar to the ability of E1A to target tissue-specific factors in other tissues, such as muscle.

MATERIALS AND METHODS

Plasmid constructs

Construction of the apoAI–chloramphenicol acetyltransferase (CAT) reporter plasmids –256AI.CAT, –192AI.CAT, –133AI.CAT, –256/–80AI.CAT, –256/–192AI.CAT, –222/–110AI.CAT, 2×[–241/–192].AI.CAT, –2500AI.CAT and –41AI.CAT have been described previously (4,5,8). The –178/–148AI.CAT, –178/–154AI.CAT and –196/–174AI.CAT reporters were constructed by subcloning double-stranded oligonucleotides corresponding to these regions of the apoAI promoter into the *Bam*HI site of –41AI.CAT. The –220/–135AI.Luc reporter (AB.Luc) has been previously described (11). The E1A 12S and 13S expression vectors (47) were provided by Dr Joseph Nevins. The 12S mutant expression vectors (26,33,39) were provided by Dr Elizabeth Moran. The Egr-1 expression vector pCMV5-Egr-1 (48) was provided by Dr Vikas Sukhatme. The transthyretin TTR 12×HNF3.CAT reporter, HNF3β and HNF3α expression vectors (49,50) were provided by Dr Robert Costa. The HNF4 expression vector (51) was provided by Dr Frances Sladek. The CBP expression vector (42) was provided by Dr Richard Goodman. The pRb expression vector (52) was provided by Dr Frederic Kaye. The E2F–CAT reporter constructs were provided by Srilata Bagchi (37). The E2F expression vectors were provided by Dr Ali Fattaey (53).

Tissue culture and transient transfection assays

HepG2 cells, maintained as previously described (5), were co-transfected using the calcium phosphate co-precipitation method (4) with CAT or luciferase reporter plasmids plus either pRSVβ-gal or pCMVβ-gal, to control for DNA uptake, as indicated in the figure legends. Cell extracts were prepared 20 h following transfection and assayed for CAT, luciferase or β-galactosidase activities as previously described (10,54). Data shown were derived from two or three independent experiments.

Western analysis

Nuclear protein extracts prepared from HepG2 cells transiently transfected with HNF3β and 12S E1A expression vectors were resolved by SDS–PAGE on 8% polyacrylamide gels. Protein was transferred to nitrocellulose and analyzed by Western analysis as

previously described (55) using serum against HNF3β (a gift of Dr Robert Costa) or an anti-E1A antibody (Santa Cruz Biotechnology).

Gel retardation assays

Nuclear extracts were prepared from HepG2 cells as described (56). A double-stranded oligonucleotide containing nucleotides –178 to –154 (oligo B) of the apoAI enhancer (5) was used for gel mobility shift assays as described (57). E1A 13S protein was translated using T3 RNA polymerase and the TnT reticulocyte lysate system (Promega). Bacterially produced E1A 13S protein was purchased from Santa Cruz Biotechnology.

RESULTS

E1A represses apoAI promoter activity in HepG2 cells

To determine the effect of E1A on the transcriptional activity of the apoAI promoter the –2500AI.CAT reporter, which contains 2500 nt of the 5′-flanking region of the apoAI gene (4), was co-transfected with increasing amounts of an E1A 13S expression vector into HepG2 cells. The reporter activity was inhibited 4-, 5.3- and 13.6-fold by 0.02, 0.05 and 0.1 μg respectively of the E1A 13S expression vector (Fig. 1A). A control vector not containing E1A had no effect on apoAI reporter activity (Fig. 1A). To define the promoter elements required for this repression, reporter constructs containing various deletions in the –256 to –41 region of the apoAI promoter were co-transfected with the E1A 13S expression vector into HepG2 cells (Fig. 1B). The –256AI.CAT construct, containing enhancer elements A, B and C (5), was repressed 8-fold by the E1A 13S expression vector. The –192AI.CAT construct, containing sites B and C, was repressed 6-fold by E1A 13S. Repression of apoAI promoter activity by E1A 13S was lost when the region between nt –192 and –133 was deleted in constructs –133AI.CAT and –256/–192AI.CAT. Sites B and E1 are located within this region of the apoAI enhancer (5,12). The activities of a series of apoAI reporters containing progressively smaller regions of the apoAI enhancer (–222/–110AI.CAT, –203/–140AI.CAT and –178/–148AI.CAT) were all repressed 4-fold by E1A 13S. The –178/–148 region contains only apoAI enhancer site B, consisting of two HNF3β binding sites (10). Finally, the activity of construct –178/–154AI.CAT, which contains only one of these HNF3 sites, was also repressed 3-fold by E1A 13S, suggesting that a single HNF3 binding site is adequate to mediate E1A 13S repression.

Activation of the apoAI promoter by HNF3β and HNF3α through site B is inhibited by E1A

The location of the *cis*-acting elements within the apoAI enhancer are shown in Figure 2A. Construct –178/–154AI.CAT, containing only site B (site B reporter), has low activity in HepG2 cells relative to constructs containing additional enhancer elements (4,5). Co-transfection of HNF3β or HNF3α expression vectors with the site B reporter induced its activity 4-fold (Fig. 2B). This transcriptional induction was inhibited by co-expression of E1A 13S. The low basal activity of the site B reporter, due to endogenous HNF3 in HepG2 cells (10), was also repressed by E1A 13S. Additional studies showed that, consistent with previous work (9), the activity of a construct controlled by two copies of site A, 2×[–241/–192].AI.CAT, was increased 4-fold when co-transfected with a HNF4 expression vector. However, this induced expression was not affected by co-expression of E1A
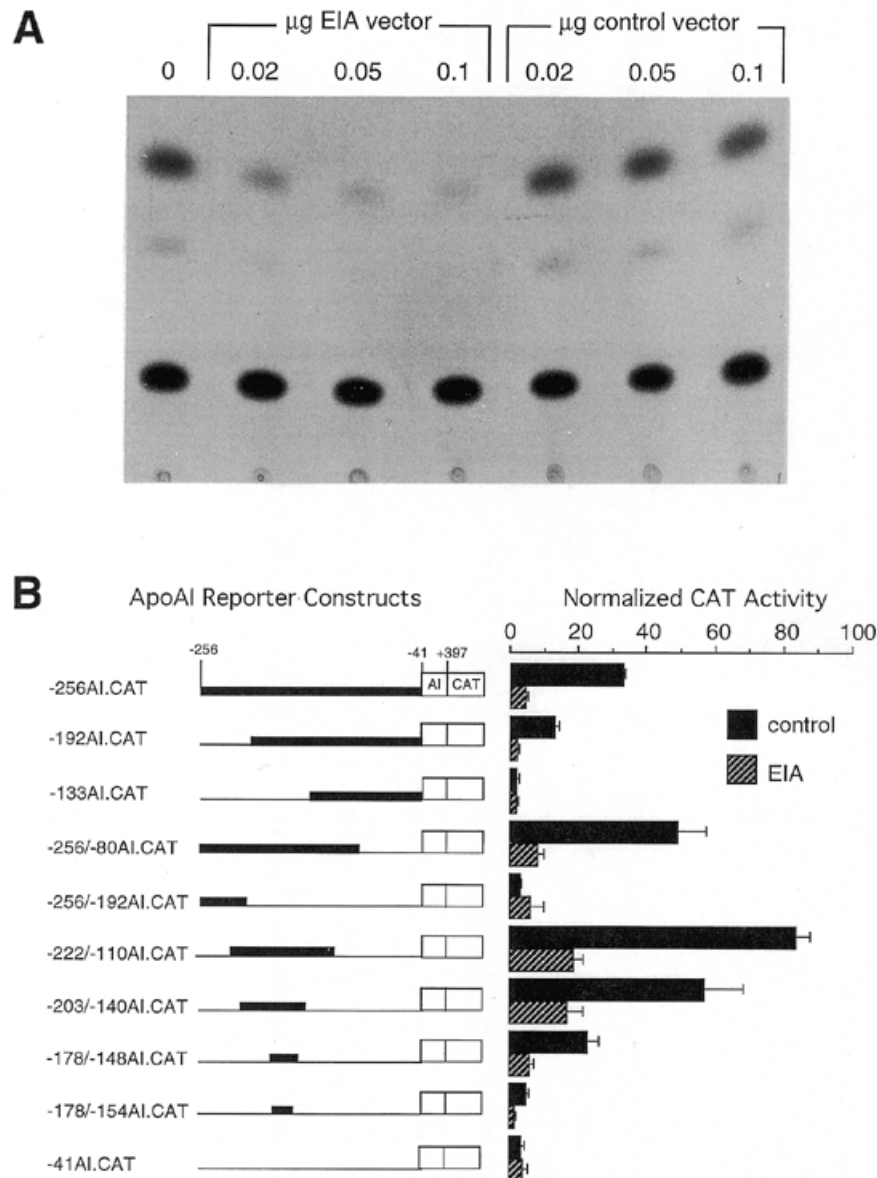
Figure 1. Indentification of apoAI promoter elements involved in E1A-mediated repression. (**A**) The –2500AI.CAT reporter (8 µg) was co-transfected with the RSV-β-galactosidase (β-gal) expression vector (2.0 µg) and the indicated amounts of either an E1A 13S expression vector or a control vector containing no insert. CAT activity was determined in cell extracts normalized for β-gal activity. A representative CAT assay is shown. (**B**) CAT reporter constructs (8 µg) under transcriptional control of the apoAI promoter (–256AI.CAT) or the indicated deletion mutants were co-transfected into HepG2 cells with the β-gal expression vector (2 µg) in the absence (filled bars) or presence (hatched bars) of the E1A 13S expression vector (0.2 µg). CAT activity was determined, normalized to β-gal and the obtained values used to generate the bar plots shown.

13S (Fig. 2B). Similarly, the activity of a construct containing the E1 element alone, construct –196/–174AI.CAT, was induced 3-fold when co-transfected with an Egr-1 expression vector but this induced activity was also not affected by co-expression of E1A 13S (Fig. 2B). Together, these results suggest that E1A selectively inhibits HNF3 activation of the apoAI promoter.

HNF4 and HNF3 bind to the apoAI enhancer sites A and B, respectively, and synergize to activate apoAI transcription (5,10). This synergy can be observed using a luciferase reporter controlled by sites A, E1 and B (–220/–135AI.Luc; Fig. 2B). Co-transfection of this construct with either HNF3 or HNF4 expression vectors resulted in transcriptional induction levels which when added together were significantly lower than the

level obtained when both HNF3 and HNF4 expression vectors were co-transfected together (Fig. 2B). However, expression of E1A 13S repressed activity of the –220/–135AI.Luc construct to approximately the same extent when either the HNF3β expression vector alone or both the HNF3β and HNF4 expression vectors were co-transfected (Fig. 2). Thus although HNF4 can synergistically enhance HNF3β activation of the apoAI promoter, this synergy is insufficient to prevent E1A-mediated repression of HNF3 activity.

To determine whether E1A inhibition of HNF3 activity was dependent on the specific response element used (i.e. site B) E1A effects on a reporter controlled by the HNF3 response element in the transthyretin (TTR) promoter (49,58,59) were also investigated. The HNF3 binding site between nt –111 and –85 of the TTR
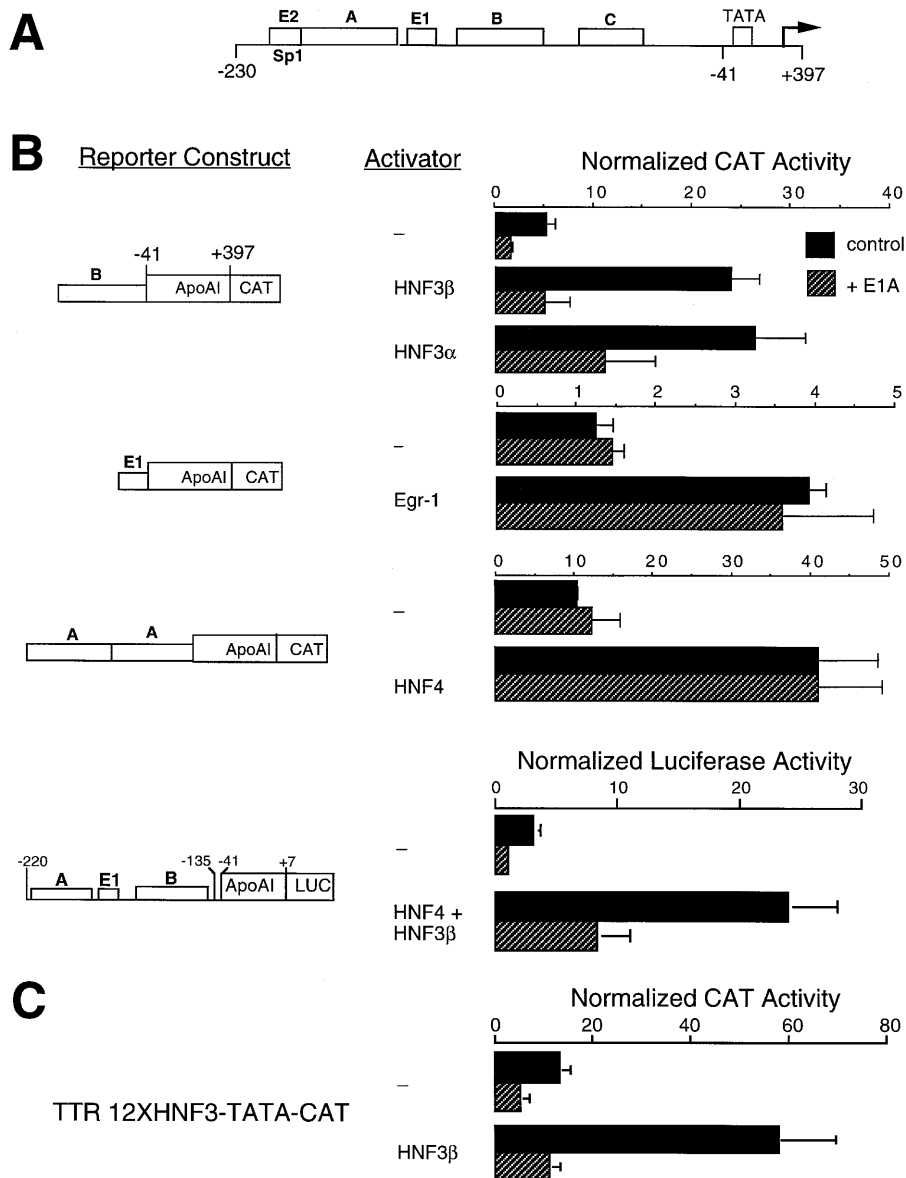
Figure 2. E1A 13S inhibits HNF3β transcriptional activity. (**A**) The location of the apoAI enhancer elements A, B, C, E1 and E2/Sp1 are shown. (**B**) HepG2 cells were co-transfected with the reporters and activators indicated along with the E1A 13S and β-gal expression vectors as described in Figure 1. The amounts of co-transfected activator plasmids were: HNF3β, 0.2 μg; Egr-1, 0.5 μg; HNF4, 1.0 μg. CAT activity in cell extracts was normalized to β-gal. The presence or absence of the E1A 13S expression vector is indicated by hatched and solid bars respectively. (**C**) The TTR 12×HNF3 reporter was co-transfected with the expression vectors indicated and its activity determined as described above.

promoter has only 36% identity to apoAI site B (49). A reporter containing 12 copies of the TTR promoter HNF3 binding site (TTR 12×HNF3.TATA.CAT) was induced 4-fold by co-transfection with the HNF3β expression vector and this induced activity was efficiently repressed by E1A 13S (Fig. 2C). Since E1A inhibition of HNF3 transcriptional activity was independent of the specific sequence of the HNF3 response element, E1A may inhibit expression of numerous genes with promoters containing HNF3 binding sites.

Dominance of E1A repression on HNF3 activity

Inhibition of HNF3 activity by E1A could be due to an effect on HNF3 itself or to E1A partitioning of a limiting common factor

required for HNF3 transcriptional activity. To distinguish between these possibilities, HepG2 cells were co-transfected with the site B reporter plus a limiting amount of E1A expression plasmid (sufficient to inhibit activity by 80%) and increasing amounts of the HNF3β expression vector (Fig. 3A). Again, the basal activity of the reporter due to endogenous HNF3 was inhibited ~3-fold by E1A. The reporter activity was increased by increasing amounts of the HNF3β expression vector, with maximal activation achieved when 0.2 or 1.0 μg HNF3β expression was co-transfected. Although 1.0 μg transfected HNF3β expression vector was above the amount required for maximal activation of reporter activity, E1A was still able to repress reporter activity when this amount of HNF3β expression vector was used. Western analysis indicated that transfection of the HNF3β expression vector resulted in a
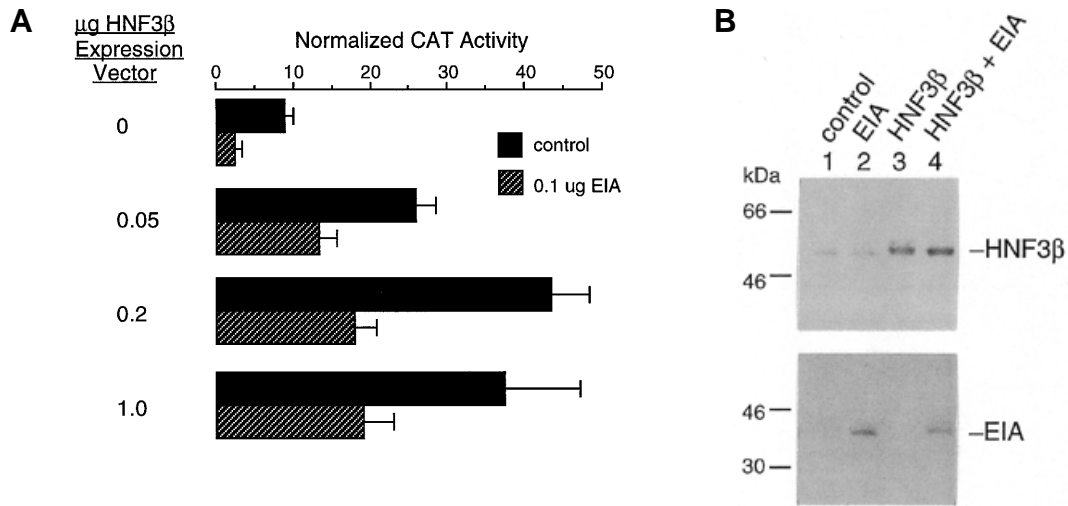
Figure 3. Dominant repression of HNF3 by E1A. (**A**) HepG2 cells were co-transfected with the site B reporter (8 µg), the E1A 13S expression vector (0.1 µg), the β-gal expression vector (2 µg) and different amounts of the HNF3β expression vector as indicated. CAT activity in cell extracts was normalized for β-gal activity. Transfections in the absence or presence of the E1A 13S expression vector are shown as solid or hatched bars respectively. (**B**) Nuclear extracts prepared from HepG2 cells transfected with empty expression vector (lane 1) or 0.2 µg expression vectors encoding E1A 12S (lane 2), HNF3β (lane 3) or E1A and HNF3β (lane 4) were analyzed by Western analysis for levels of HNF3β (upper) and E1A protein (lower).

several-fold increase in HNF3β protein levels (Fig. 3B, upper panel), correlating with the increased transcriptional activity of the site B reporter. There was no effect of E1A expression on either basal HNF3β levels or on the elevated HNF3β levels present in co-transfected cells (Fig. 3B). Thus HNF3β overexpression was unable to overcome repression by E1A.

One mechanism by which E1A could inhibit HNF3 activity is by E1A-mediated inhibition of HNF3 binding to DNA. This possibility was evaluated by electrophoretic mobility shift assays (EMSA) using a ^{32}P-labeled oligonucleotide spanning the –178 to –154 region of site B (Fig. 4). Protein–DNA complexes containing HNF3α or HNF3β from HepG2 cell nuclear extracts and oligo B were identified by supershift using HNF3α and HNF3β specific antibodies (Fig. 4, lanes 3 and 4). HNF3γ protein–DNA complexes were not detected using HNF3γ-specific antibody (Fig. 4, lane 5). Nuclear extracts prepared from HepG2 cells (lane 6) or from HepG2 cells transfected with the E1A expression vector (Fig. 7) showed identical intensities of HNF3 shifted complexes. The percentage of cells transfected in these experiments was undetermined, so it remained possible that the presence of HNF3 from a large number of transfected cells overwhelmed the E1A present in the nuclear extracts. However, addition of large amounts of *in vitro* translated E1A or bacterially expressed E1A 13S (0.5 µg) to the binding reactions also did not influence the ability of either HNF3α or HNF3β to bind site B (Fig. 4, lanes 8–11). Finally, there was no difference in mobility between the HNF3 complexes formed in the absence or presence of E1A generated in either mammalian cells, reticulocyte lysate or bacteria. Together these results argue for a model whereby E1A targets and inactivates a limiting factor required for optimal activity of HNF3β in HepG2 cells.

CBP and pRb are not HNF3β coactivators

The cellular proteins CBP and pRb are present in limiting amounts in cells, function as coactivators for various transcription factors and are inhibited by E1A (21,23,30,41,42,60). We
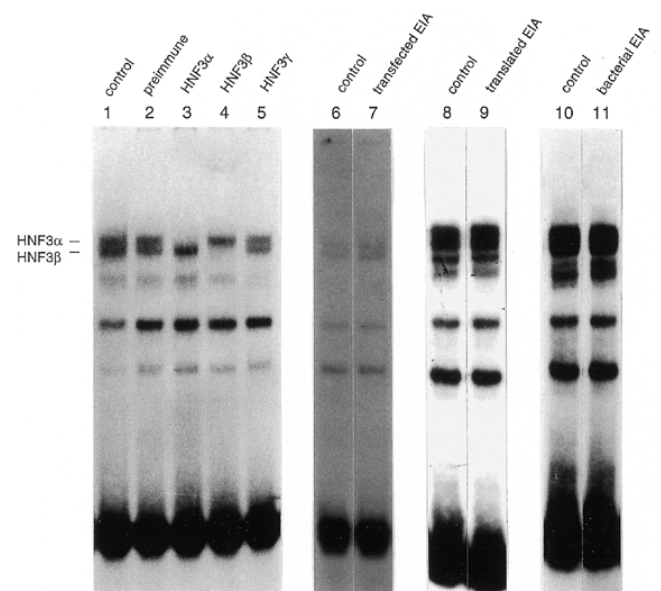

Figure 4. Effects of E1A on the DNA binding properties of HNF3. Nuclear extract prepared from HepG2 cells (10 µg, lanes 1–6 and 8–11) or HepG2 cells transfected with E1A expression vector (lane 7) were preincubated for 10 min with no addition (lanes 1–8 and 10) or with 0.5 µl *in vitro* translated 13S E1A (lane 9) or 0.5 µg bacterially expressed E1A (lane 11). A radiolabeled double-stranded oligonucleotide spanning the apoAI promoter site B (nucleotides –178 to –154) was added and the incubation was continued for 10 min on ice. Preimmune serum (lane 2) or serum specific for HNF3α (lane 3), HNF3β (lane 4) or HNF3γ (lane 5) was subsequently added and the mixture was incubated on ice for 15 min. The protein–DNA complexes were resolved by electrophoresis on a 5% non-denaturing polyacrylamide gel. HNF3α- and HNF3β-specific complexes are indicated.

determined whether CBP or pRb function as HNF3 coactivators and whether their inactivation by E1A could account for E1A-mediated inhibition of HNF3β by asking whether overexpression of these
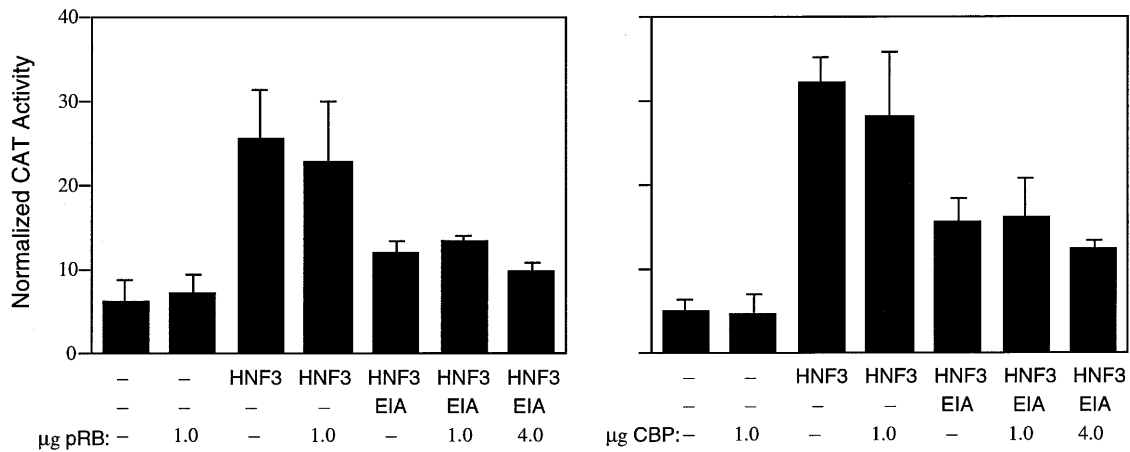
Figure 5. pRb and CBP are not involved in the E1A-mediated inhibition of HNF3. HepG2 cells were transfected with the site B reporter (8 μg) and expression vectors for HNF3β (0.2 μg), E1A 13S (0.2 μg), β-gal (0.5 μg), pRb (1.0 or 4.0 μg) and CBP (1.0 or 4.0 μg) as indicated. CAT activity in cell extracts was normalized to β-gal and the obtained values were used to generate the bar plots shown.
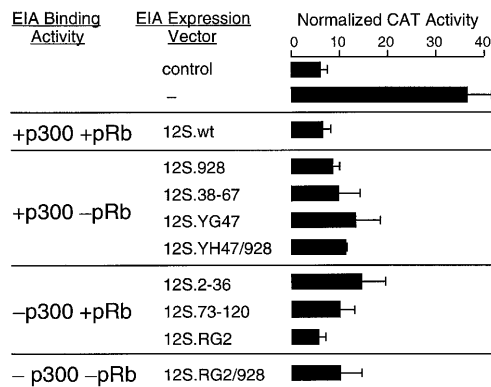
Figure 6. E1A domains involved in HNF3β repression. HepG2 cells were co-transfected with the site B reporter (8 μg), the HNF3β expression vector (0.2 μg), the CMV-β-gal expression vector (2 μg) and the indicated E1A 12S mutant expression vectors (4 μg). CAT activity in cell extracts was normalized to β-gal. Control denotes reporter activity in the absence of HNF3β and E1A.
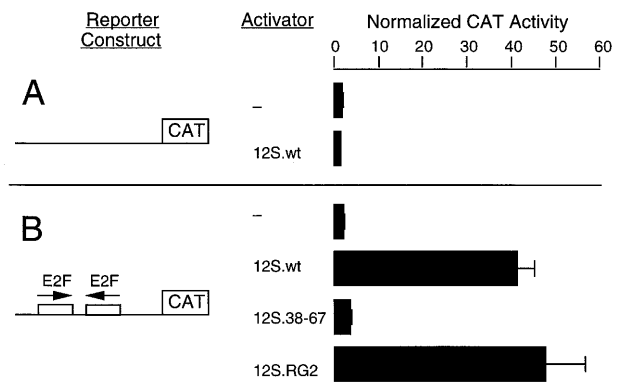
Figure 7. E1A activation of E2F reporter activity. HepG2 cells were co-transfected with a control CAT reporter (**A**) or a reporter containing two copies of an E2F response element (**B**) and the CMV-β-gal expression vector (2 μg). Addition of the wild-type (12S.wt) or 12S.38–67 E1A mutant expression vector (0.5 μg) is indicated. – denotes reporter activity in the absence of E1A expression vectors. CAT activity in cell extracts was normalized to β-gal.

factors could reverse E1A-mediated inhibition of HNF3β. The site B reporter was activated with HNF3 in the presence of an inhibitory amount (0.2 μg) of E1A expression vector and various amounts of CBP or pRb expression vectors were tested for their ability to reverse E1A-mediated inhibition. Transfection of pRb or CBP neither increased site B reporter activity due to endogenous HNF3 nor increased HNF3β activity in co-transfected cells (Fig. 5). Control experiments (data not shown) showed that CBP enhanced the activity of the estrogen and RARα receptors as described previously (21–23). Further, at all concentrations tested neither pRb nor CBP reversed E1A-mediated repression of HNF3β (Fig. 5).

The regions of E1A required for interaction with pRb or CBP include the N-terminal region (residues 1–39), the CR1 region (residues 40–80) and the CR2 region (residues 120–139) (reviewed in 27). Expression vectors encoding wild-type E1A 12S or E1A 12S containing mutations in these regions (26,33,39) were tested for their ability to inhibit HNF3 activity. In co-transfection experiments using the site B reporter and the HNF3β expression vector the wild-type E1A 12S and the mutant E1A 12S constructs all efficiently repressed HNF3β transcriptional activity (Fig. 6). In control experiments the ability of the E1A mutant constructs to interact with pRB-related proteins was verified by testing their effects on E2F CAT reporter activity. Expression of wild-type E1A 12S (12S.wt) increased E2F reporter activity 20-fold, while the E1A 12S construct containing a mutation within the CR1 region (12S.38–67) that was previously shown to interfere with the ability of E1A to release E2F proteins from pRb (32,33,61) did not significantly activate the E2F reporter (Fig. 7). The E1A 12S.RG2 construct, containing an N-terminal mutation that disrupts binding to p300 but not to pRb (27), was able to increase E2F reporter activity (Fig. 7). In summary, mutations in the known functional regions of E1A protein did not effect its ability to inhibit HNF3 transcriptional activity. Furthermore, pRb and CPB do not function as HNF3 coactivators and their inhibition by E1A does not account for E1A-mediated inhibition of HNF3β.

DISCUSSION

In mammals the apoAI gene is expressed predominantly in liver and intestine. Although the mechanisms responsible for this tissue specificity have not been completely elucidated, it is clear that multiprotein complexes containing both DNA-bound transcription factors and factors not directly bound to DNA play a fundamental role (5,11). In this study we used the adenovirus 12S and 13S early region proteins E1A as molecular probes (27) to perturb these multiprotein complexes. Expression of these proteins repressed apoAI enhancer activity in HepG2 cells. Site B within the enhancer was identified as the *cis*-acting element involved in E1A-mediated repression. Activation of site B by overexpression of HNF3β or HNF3α was potently repressed by E1A. Similar activation of a structurally different HNF3 response element derived from the TTR promoter (49) was also repressed by E1A. In contrast, activation of apoAI enhancer sites A or E1 by HNF4 or Egr-1, respectively, was resistant to repression by E1A. Finally, E1A did not alter levels of HNF3β in nuclear extracts and did not inhibit formation of HNF3–DNA complexes in mobility shift assays. Taken together these observations suggest that E1A-mediated repression of the apoAI enhancer is due to selective inactivation of HNF3 interaction with a required cofactor.

Recent studies suggest that p300/CBP represents a family of common factors required for transcriptional activity of nuclear receptors (21–24) and several other transcription factors, such as CREB, AP1 (60,62) and Stat2 (63). Inactivation of p300/CBP by E1A inhibits the transcriptional activity of nuclear receptors (21). In the present study overexpression of CBP did not reverse E1A-mediated inhibition of HNF3β, suggesting that partitioning of limiting amounts of p300/CBP by E1A does not explain E1A-mediated inhibition of HNF3 in HepG2 cells. E1A also targets pRb, p107 and p130, collectively known as 'pocket' proteins (reviewed in 27,31,32). Interaction of E1A with 'pocket' proteins releases the 'pocket' protein-associated transcription factor E2F (30,33,37). However, overexpression of pRb failed to reverse E1A-mediated inhibition of HNF3β. Further, mutations in E1A known to inhibit interactions with 'pocket' proteins and the p300/CBP coactivators did not interfere with E1A-mediated inhibition of HNF3. These data indicate that interaction of E1A with 'pocket' proteins and with p300/CBP proteins are not essential for E1A-mediated inhibition of HNF3.

It has recently been shown that E1A 12S represses estrogen receptor (ER)-dependent transcription and that this repression is reversed by co-expression of CBP (21). However, full functionality of CBP in this assay depends on the presence of SRC or SRC-like coactivator proteins bound to the ER–CBP protein complex (23). It is therefore conceivable that inactivation of a limiting complex containing CBP and an HNF3 coactivator by E1A is responsible for E1A-mediated inhibition of HNF3 and that both components in this complex will be required to reverse E1A-mediated inhibition of HNF3. In contrast to other nuclear receptors (21,22), HNF4 was not inhibited by E1A. Since E1A-mediated inhibition of nuclear receptors is thought to be due to inactivation of p300/CBP, a common coactivator for several nuclear receptors (reviewed in 24), this observation suggests that p300/CBP is not a cofactor for HNF4.

In certain cases E1A binds directly and inactivates transcription factors. For example, direct interaction of the N-terminal domain of E1A with the bHLH domains of the myogenic determination transcription factors myogenin and E12 correlates with E1A-mediated inhibition of myogenesis and repression of muscle-specific gene expression (43). However, experiments attempting to show direct protein–protein interactions between E1A and HNF3 indicated that E1A does not prevent HNF3 binding to DNA nor does it directly interact with HNF3β, as determined by electrophoretic mobility shift assays. E1A can inhibit the myogenic activity of bHLH proteins by several independent mechanisms, including direct protein–protein interaction (43), inhibition of the myogenic coactivator activity of CBP (45) and pRb (44) and indirectly by phosphorylation of MyoD resulting from loss of p21 (Cip1/WAF1) regulation of cyclin D1 activity (64,65). In cardiac myocytes tissue-specific transcription is repressed by expression of E1A proteins containing mutations in either the CBP or the 'pocket' protein binding domains (46). In summary, even though E1A is a potent inhibitor of HNF3, none of the previously established mechanisms for E1A-mediated transcriptional repression seem to explain E1A-mediated inhibition of HNF3.

HNF3 occupies a primary position in the hierarchy of developmental signals that culminate in endoderm differentiation and liver development (reviewed in 66). The existence of endogenous E1A-like factors in embryonal carcinoma cells (18) and the finding in the current study that E1A regulates HNF3 activity suggests that E1A-like factors may play an important role in liver development. In addition, these findings suggest that modulation of HNF3 activity by E1A-like factors in liver could play an important role in regulation of apoAI gene expression.

ACKNOWLEDGEMENTS

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