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Promoter-Specific *trans*-Activation by the Adenovirus $E1A_{12S}$ Product Involves Separate E1A Domains

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Recent studies have shown that the adenovirus $E1A_{12S}$ product can *trans*-activate transcription by activating the transcription factor E2F. However, E2F cannot be the only target for the $E1A_{12S}$ product, since several cellular promoters have been found to be activated by the $E1A_{12S}$ protein even though they lack E2F sites. Indeed, we now show that activation of the *hsp70* promoter by the $E1A_{12S}$ product requires the TATAA sequence. Moreover, activation of the *hsp70* promoter requires the N-terminal domain of the E1A protein and does not require the conserved region 2 sequences which are required for the E2F-dependent activation of transcription. We conclude that the targeting of distinct transcription factors, leading to *trans*-activation of transcription of multiple promoters, involves distinct domains of the E1A proteins that are also required for oncogenic activity.

The adenovirus E1A gene encodes proteins that are essential for replication of the virus (4, 24). A variety of experiments have demonstrated that the primary function of E1A in this regard is activation of the early viral transcription units (4, 24, 40). Since the E1A products are not DNA-binding proteins (12), this activation of transcription must be indirect, making use of host cell components. Indeed, it is clear that multiple cellular transcription factors are utilized in the process of E1A-mediated activation of viral transcription (41).

The E1A gene is a complex transcription unit that encodes multiple protein products. Distinct mRNAs are generated through the alternative splicing of a single pre-mRNA molecule. The two largest mRNAs, termed $E1A_{13S}$ and $E1A_{12S}$, are the predominant species and appear to be sufficient for the lytic cycle of the virus. Comparison of a large number of adenovirus serotypes shows that three regions of E1A coding sequence are more conserved than the remaining regions (26). Two of these regions, designated conserved regions 1 and 2 (CR1 and CR2), are found in both the $E1A_{12S}$ and $E1A_{13S}$ mRNAs, whereas the CR3 sequence is unique to the $E1A_{13S}$ mRNA. Analysis of a large number of mutants that affect E1A function has demonstrated that the CR3 sequence is critical for the activation of early viral transcription (31, 36, 48).

Although the primary function of E1A is to facilitate lytic growth, it is also true that E1A expression can have oncogenic effects when conditions for lytic growth are not permissive. Expression of E1A alone can confer proliferative capacity to a cell that otherwise would not grow continuously (20), and in conjunction with the adenovirus E1B gene or an activated *ras* gene, E1A can transform cells to an oncogenic state (51). Once again, the analysis of a large series of E1A mutants has defined the sequences that are essential for this function (15, 24, 30, 36, 52, 57, 60, 63). In contrast to the results of *trans*-activation assays, sequences within the $E1A_{12S}$ product are sufficient for transformation, and mutations within the CR3 sequences generally have little or no effect on transformation. On the basis of these results, it has generally been argued that the major *trans*-activation function of E1A is carried out by the $E1A_{13S}$ product, dependent on CR3 sequence, while the ability of E1A to immortalize cells or transform in collaboration with other oncogenes is independent of CR3.

An elucidation of the mechanism by which E1A functions as an oncogene is clearly an important goal. Several reports have suggested a role for the $E1A_{12S}$ product in the repression of transcription, although this activity has not been characterized in great detail (5, 23, 30, 43, 50, 58, 59, 62). Other studies have shown that the $E1A_{12S}$ product can *trans*-activate transcription, albeit with reduced efficiency compared with that of the $E1A_{13S}$ product and usually with a more limited specificity (29, 56, 66, 68). Recent experiments have more clearly focused these studies and have now provided a clear target for the *trans*-activation by the $E1A_{12S}$ product and in so doing have defined a biochemical mechanism for the activity. Transcription factor E2F, an essential component for transcription of the adenovirus E2 promoter (27), normally complexes with a 19-kDa product of the adenovirus E4 gene, which then allows cooperative binding to the E2 promoter (16, 21, 34, 39, 47). In most cell types, E2F was found to exist in complexes with other cellular proteins, preventing the interaction of E4 with E2F (1). The $E1A_{12S}$ protein can dissociate these complexes, releasing free E2F that can then interact with E4 (1). Moreover, conditions that promote the release of E2F from the cellular complexes result in a stimulation of E2F-dependent transcription, even in the absence of E4. These results suggest that the interaction of E2F with cellular factors is regulatory, possibly preventing E2F transcriptional function.

Within the $E1A_{12S}$ product, sequences at the extreme N terminus, sequences within CR1, and sequences within CR2 are all required for oncogenic activity (15, 23, 30, 36, 52, 57, 60, 63). The CR1 and CR2 sequences have been shown to be necessary for binding to a variety of cellular proteins such as the retinoblastoma gene product and cyclin A (13, 44, 63) and more recently have been shown to be involved in E2F dissociation and *trans*-activation of an E2F-dependent pro-

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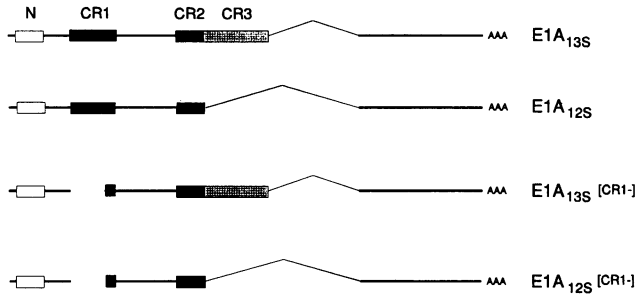

FIG. 1. $E1A_{13S}$ and $E1A_{12S}$ cDNA expression constructs (CMV_{13S} and CMV_{12S}). The wild-type $E1A_{13S}$ and $E1A_{12S}$ cDNAs, which produce mRNAs encoding the 289- and 243-aa E1A proteins, respectively, are depicted at the top. The three regions of conserved sequence, CR1 (aa 41 to 77), CR2 (aa 121 to 138), and CR3 (aa 140 to 188), as well as the N-terminal sequence that is functionally important are indicated. Shown below are the $E1A_{13S}$ and $E1A_{12S}$ cDNAs with a deletion, Δ38-67, in the CR1 region (CMV_{13S}[CR1-] and CMV_{12S}[CR1-]).

moter (1, 46). Sequences at the N terminus are also involved in the binding to at least one cellular protein, a 300-kDa polypeptide (11, 59, 64). Given the role of proteins that interact with CR1 and CR2 in transcriptional control by $E1A_{12S}$, we have investigated the possible role of interactions at the N terminus. We find that the cellular *hsp70* promoter is activated by $E1A_{12S}$, dependent on the TATAA element, and that this activation is dependent on the N-terminal sequences of E1A.

MATERIALS AND METHODS

Plasmid structures and constructions. The $E1A_{13S}$ and $E1A_{12S}$ cDNA expression constructs depicted in Fig. 1 (CMV_{13S} and CMV_{12S}) were generated by inserting the *Hin*dIII-*Pst*I fragment from pSVHpE1A$_{13S}$ or pSVH pE1A$_{12S}$ (36) between the *Hin*dIII-*Pst*I sites of $pGem_1$ to form $pGem_1$ E1A$_{13S}$ or E1A$_{12S}$. The *Hin*dIII-*Bam*HI fragment from the $pGem_1$E1A construct was then inserted between the *Hin*dIII and *Bam*HI sites of the expression vector pBC12 (9) to form the CMV_{13S} and CMV_{12S} plasmids. The CMV_{12S} [CR1-] construct was created by polymerase chain reaction, using pE1A 38-67 (58) as the template and primers designed to create a 5′ end perfectly matched to the CMV_{12S} and CMV_{13S} constructs (i.e., the E1A sequence starts at 55 nucleotides prior to the translation start site). CMV_{13S} [CR1-] was created by subcloning a *Bst*XI fragment encompassing the CR1 deletion from CMV_{12S} [CR1-] into the *Bst*XI sites of CMV_{13S} to form CMV_{13S} [CR1-].

Adenovirus early-gene promoter constructs pE2-CAT (+40/−85) (32), pE3-CAT (+31/−237) (67), and pE4-CAT (+32/−224) (42) have been described previously. The wild-type *hsp70*-chloramphenicol acetyltransferase (CAT) construct (pHC-WT or pHC-1170) and TATA mutants (pHC-pm5 and pHC-pm6) have been described previously (52).

Several of the $E1A_{12S}$ expression constructs depicted in Fig. 4A were described previously (59). These E1A plasmids consist of *Eco*RI-*Pst*I fragments (including the E1A promoter) cloned into pUC118. They are named for the amino acids deleted. pmHN3 contains a single-point mutation resulting in an H-to-N change at amino acid (aa) 3; pmDA21 contains a single-point mutation resulting in a D-to-A change at aa 21; pm928 contains a single-point mutation resulting in a C-to-G change at aa 124; pm961 contains a single-point mutation resulting in a Q-to-K change at aa 135; pm928/961 is a double-point mutant with changes at both aa 124 and 135.

Transfection and CAT assays. CV-1 cells were transfected by the calcium phosphate procedure. Cells were plated on 10-cm-diameter dishes and received a total of 25 μg of DNA per plate. Cells were washed 16 h later and were refed and harvested 48 h after transfection. CAT assays were performed as described by Gorman et al. (14).

Immunoprecipitation and Western immunoblotting. Transfected cells were lysed in 250 mM NaCl–0.1% Nonidet P-40, 50 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES; pH 7.0). Lysates were frozen in liquid N_2, thawed on ice, and spun in a microcentrifuge at 13,000 rpm for 3 min. Supernatants were incubated with 2 μl of the anti-E1A monoclonal antibody M73 (recognizing the E1A carboxy terminus) for 1 h at 4°C (17). Immune complexes were collected on protein A-Sepharose (Pharmacia), washed, and suspended in sodium dodecyl sulfate (SDS) sample buffer (2% SDS, 100 mM dithiothreitol, 10% glycerol, 0.1% bromophenol blue, 50 mM Tris [pH 6.8]). The samples were boiled for 5 min and separated on an SDS–10% polyacrylamide gel. Samples were transferred to nitrocellulose, using an LKB 2117-250 Novablot electrophoresis unit at 135 mA for 2 h. E1A proteins were detected by using the M73 antibody. Immune complexes were detected with goat anti-mouse immunoglobulin G-alkaline phosphatase conjugate, using 5-bromo-4-chloro-3-indolylphosphate *p*-toluidine salt and nitroblue tetrazolium chloride as chromagenic substrates for the enzyme alkaline phosphatase as instructed by the manufacturer (Bethesda Research Laboratories).

RESULTS

$E1A_{13S}$ and $E1A_{12S}$ function independently as *trans*-activators. Although the target specificity for activation of transcription by the $E1A_{13S}$ product has been explored in considerable detail, much less is known of the range of targets for the $E1A_{12S}$-dependent activation of transcription. Recent experiments have identified the adenovirus E2 promoter as one target, dependent on the E2F transcription factor, but to more clearly define the nature of transcription activation by the $E1A_{12S}$ product, and in particular the target specificity, we have analyzed the activation capacity of various E1A expression vectors depicted in Fig. 1. In addition to the wild-type $E1A_{13S}$ and $E1A_{12S}$ cDNAs, we have constructed altered versions of each in which sequences within CR1 have been deleted by building the Δ38-67 deletion into each of these cDNAs. Previous assays have shown that this deletion in the $E1A_{12S}$ product abolishes the ability of E1A to dissociate E2F-containing complexes and to *trans*-activate the E2 promoter (46). Thus, the $E1A_{13S}$ [CR1-] cDNA should retain the CR3 *trans*-activation function but will lack the $E1A_{12S}$-specific function, at least with respect to E2F function, thus allowing an assay of CR3-dependent activation in the absence of CR1-dependent activation.

trans-activation was measured by transfection of CAT reporter plasmids under the control of the adenovirus E2, E3, and E4 promoters into CV-1 cells along with the various E1A constructs. As shown in Fig. 2A, each of the promoters was activated by the $E1A_{13S}$ protein, consistent with the many previous assays for E1A *trans*-activation. Moreover, deletion of the CR1 sequence from the $E1A_{13S}$ construct did not impair the activation of E3 and E4. Indeed, there was a modest increase in the activation potential. In contrast, this

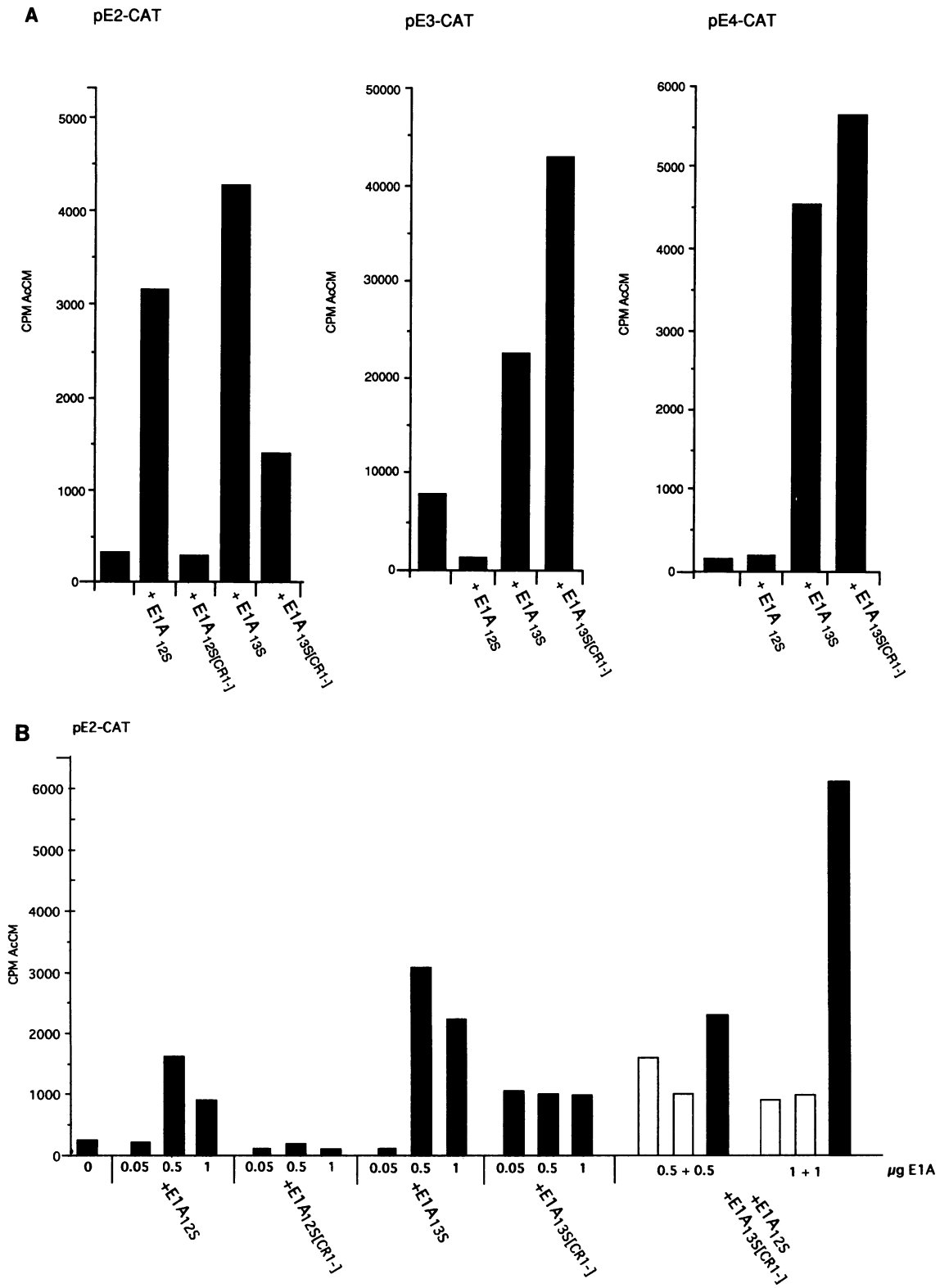

FIG. 2. *trans*-activation of early adenovirus promoters by the E1A$_{13S}$ and E1A$_{12S}$ proteins. (A) CV-1 cells were cotransfected with the E1A expression plasmids depicted in Fig. 1 (1 μg) and plasmid pE2-CAT (10 μg), pE3-CAT (5 μg), or the pE4-CAT (1 μg). CAT activity was measured in cell extracts 48 h after transfection. This level of E1A was found to be saturating. (B) CV-1 cells were transfected with plasmid pE2-CAT (10 μg) alone or in combination with the indicated E1A expression plasmids (0.05 to 1 μg). Shown as dark bars at the far right are the levels of pE2-CAT activation achieved when the two E1A expression plasmids E1A$_{12S}$ WT and E1A$_{13S}$ [CR1-] are combined in *trans*. Saturating concentrations of E1A (0.5 to 1.0 μg) were used as indicated. The open bars recapitulate that level of pE2-CAT induction seen with E1A$_{12S}$ WT and E1A$_{13S}$ [CR1-] individually, as depicted in the dark bars at the left. AcCM, acetylated chloramphenicol.

TABLE 1. Additive effect of $E1A_{12S}$ and $E1A_{13S}$ in activation of E2 transcription[a]

Plasmid	Fold induction (SE)
$E1A_{12S}$	5.4 (1.0)
$E1A_{13S}$ [CR1-]	3.5 (0.3)
$E1A_{12S}$ + $E1A_{13S}$ [CR1-]	14.0 (4.8)

[a] The $E1A_{12S}$ wild-type and $E1A_{13S}$ [CR1-] cDNAs were cotransfected separately or together along with pE2-CAT (10 μg) into CV-1 cells. The resulting levels of pE2-CAT induction and associated standard errors for three determinations are shown.

deletion reduced the capacity of the $E1A_{13S}$ protein to activate the E2 promoter.

In contrast to the broad specificity of activation by the $E1A_{13S}$ protein, the $E1A_{12S}$ protein could activate only the E2 promoter. There was no detectable stimulation of transcription from the E3 or the E4 promoter by $E1A_{12S}$ protein. Thus, it is likely that the reduced capacity of the $E1A_{13S}$ [CR1-] product to activate the E2 promoter was the consequence of the loss of the $E1A_{12S}$-specific activating function from this protein, suggesting that the $E1A_{13S}$ product is multifunctional with respect to activation of the E2 promoter. A direct demonstration was provided through the use of the $E1A_{13S}$ [CR1-] plasmid in combination with the $E1A_{12S}$ plasmid. With these plasmids, we could compare the activation mediated by either the $E1A_{12S}$ product or the $E1A_{13S}$ [CR1-] product with the activation by the two together. As shown in Fig. 2B and quantitated in Table 1, the E2 promoter was activated by either the $E1A_{12S}$ product or the $E1A_{13S}$ [CR1-] product, but the activation obtained with the two E1A plasmids together was higher than that obtained with either *trans*-activator alone. These results thus demonstrate that the mechanisms of activation mediated by the $E1A_{12S}$ product and the $E1A_{13S}$ product are different.

The *hsp70* promoter is activated by $E1A_{12S}$, dependent on the TATAA element. A series of recent reports have demonstrated that the E2F transcription factor is activated by the $E1A_{12S}$ product, consistent with the results presented in Fig. 2 showing that activation of early adenovirus promoters is restricted to the E2 promoter that contains E2F sites. Although the targets for $E1A_{12S}$ activation might be more limited than those for $E1A_{13S}$, as suggested by these results, it is also true that E2F is likely not the only target for the $E1A_{12S}$ product. Previous experiments have shown that the *hsp70* promoter (56), the proliferating cell nuclear antigen promoter (23, 37, 68), and the brain creatine kinase promoter (25) are activated by the $E1A_{12S}$ product. None of these promoters, however, are known to contain E2F-binding sites. For the *hsp70* promoter, other assays showed that activation by E1A required the TATAA element (55). In fact, the TATAA sequence appeared to be the target for activation, not just a necessary functional element, since an *hsp70* promoter that contained an alternative TATA sequence, the TATTTAT sequence found in the early simian virus 40 promoter, was functional in allowing a heat shock response but was not inducible by E1A (55). However, these assays used a genomic E1A expression vector that produced both the $E1A_{13S}$ product and the $E1A_{12S}$ product, leaving open the question as to whether the target for activation by $E1A_{12S}$ was the TATAA element.

We have assayed the previously described *hsp70* promoters that contain alterations in the TATA element in order to define the target for $E1A_{12S}$ activation of the *hsp70* promoter

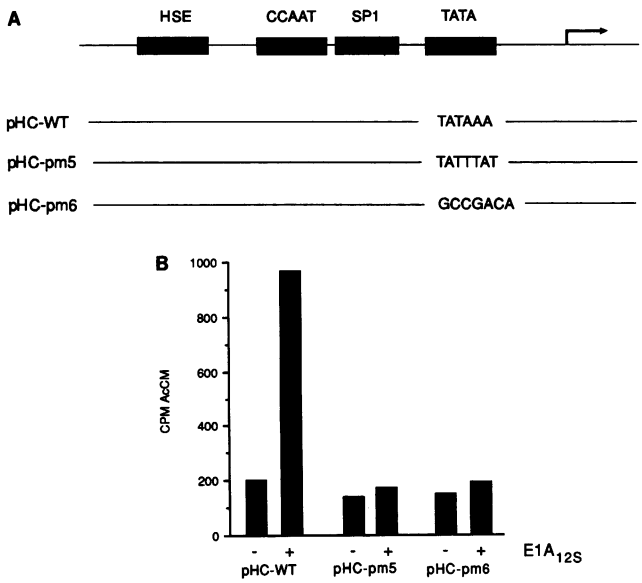

FIG. 3. Evidence that activation of the *hsp70* promoter by $E1A_{12S}$ requires the TATAA element. (A) Schematic diagrams of the wild-type *hsp70* promoter (pHC-WT) and mutants pHC-pm5 and pHC-pm6. Depicted at the top are the functional elements of the *hsp70* promoter, including the heat shock element (HSE), a CTF-binding site (CCAAT), an Sp1-binding site (SP1), and a TATA element. Shown below are the *hsp70* promoter-CAT constructs that include the wild-type TATAA sequence, the TATTTAT sequence (pHC-pm5), and the null TATA sequence (pHC-pm6). (B) Activation of the *hsp70* promoter by $E1A_{12S}$. CV-1 cells were cotransfected with the indicated hsp70-CAT constructs (5 μg) with or without the $E1A_{12S}$ WT construct (5 μg). AcCM, acetylated chloramphenicol.

(Fig. 3A). As shown in Fig. 3B, the wild-type *hsp70* promoter was indeed activated by the $E1A_{12S}$ product, consistent with previous assays using virus as the E1A source and assaying the endogenous *hsp70* gene (56). Moreover, it is also apparent from these assays that the TATAA element in the *hsp70* promoter is critical for activation by the $E1A_{12S}$ product, since the pm5 mutant, which contains the simian virus 40 TATTTAT sequence and was previously shown to be fully active in response to heat shock (55), is not activated by the $E1A_{12S}$ protein. Thus, it appears that in addition to the E2F factor, a factor that recognizes the TATAA element or that functions in concert with the TATAA element is a target for activation by the $E1A_{12S}$ product. It is also true, however, that the target cannot simply be the TATAA sequence, since this same TATAA sequence is also found in the adenovirus E3 promoter, which is not activated by the $E1A_{12S}$ product (Fig. 2). Thus, the context of the TATAA sequence element, in this case the *hsp70* promoter, must be important for the response to E1A.

Sequences at the N terminus of $E1A_{12S}$ are essential for *hsp70 trans*-activation. Our previous experiments have shown that the activation of E2F-dependent transcription by the $E1A_{12S}$ product as well as the release of E2F from heteromeric protein complexes are dependent on E1A sequences in CR1 and CR2 (1, 46). The fact that the *hsp70* promoter is activated by $E1A_{12S}$ independent of the E2F factor raised the possibility that a distinct E1A domain might be responsible for this activation. To define the requirements for activation of the *hsp70* promoter, we have analyzed a

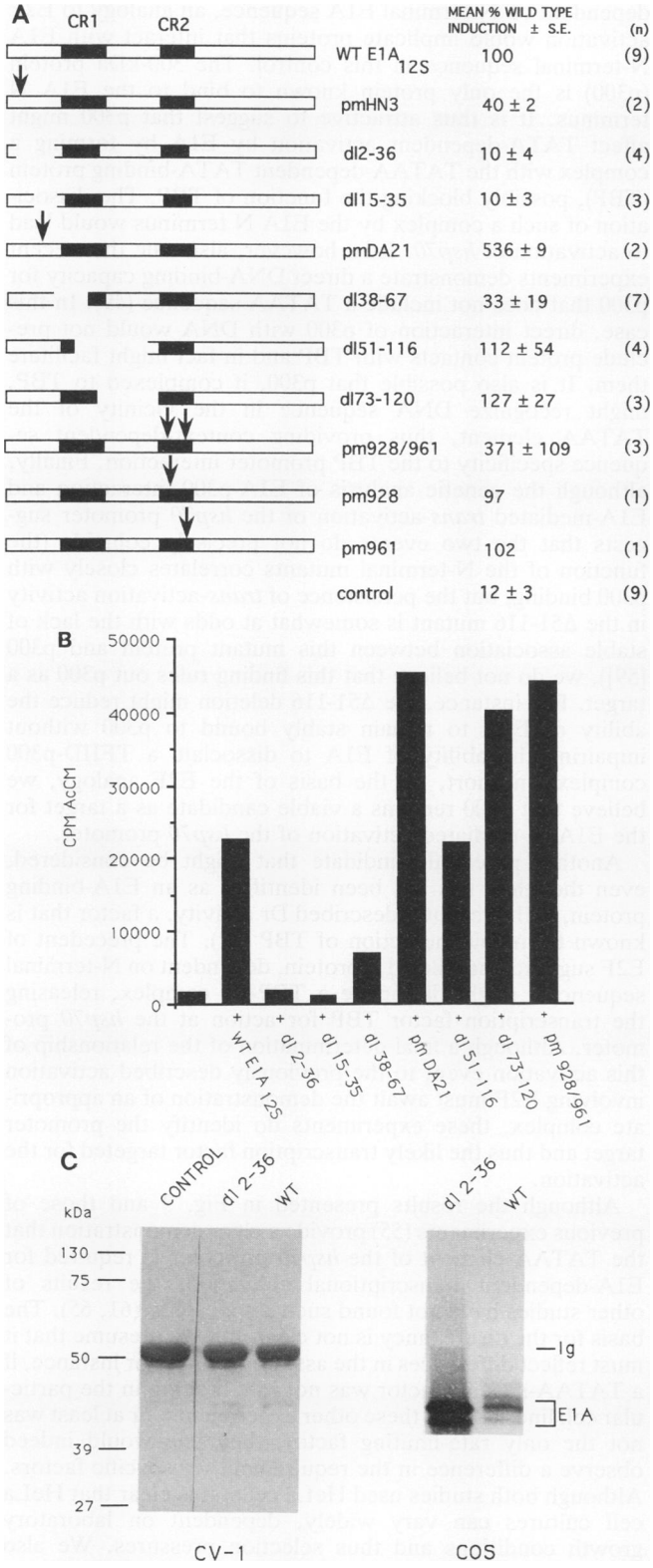

FIG. 4. Evidence that activation of *hsp70* promoter requires the N terminus of E1A. (A) Structures of the E1A$_{12S}$ mutants and results of activation assays. The wild-type 243-aa product of the E1A$_{12S}$ cDNA is depicted at the top. The two regions that exhibit sequence conservation among various adenovirus serotypes are indicated as CR1 and CR2. Point mutations are indicated by arrows, and the name of the mutant indicates the position. Deletions are depicted by gaps, and the positions are indicated in the mutant name. The mean level of induction of *hsp70* by the various E1A mutants is presented as a percentage of wild-type induction, which was set at 100%. The basal level of expression (control) averaged 6% acetylation. Cotransfection of *hsp70*-CAT with the E1A$_{12S}$ WT construct resulted in a mean eightfold activation over background. The number of independent assays of each mutant is shown as (n). Assays were performed as described below. (B) Representative transactivation assays. CV-1 cells were cotransfected with the wild-type *hsp70*-CAT plasmid (5 μg) and 5 μg of the indicated E1A$_{12S}$ mutant. Extracts were prepared at 48 h after transfection and assayed for CAT activity. AcCM, acetylated chloramphenicol. (C) Protein assays in transfected cells. Extracts were prepared from CV-1 or COS cells 48 h after transfection with pGem alone (control) or with the E1A$_{12S}$ WT (WT) or E1A$_{12S}$ Δ2-36 (dl 2-36) plasmid. E1A proteins were immunoprecipitated from the extracts and then analyzed by a Western blot assay as described in Materials and Methods. Approximate molecular masses are shown on the far left. Ig, immunoglobulin.

series of E1A mutants, each in the form of a cDNA expression vector as depicted in Fig. 4A.

The ability of each mutant protein to *trans*-activate the *hsp70* promoter was determined in a series of assays (Fig. 4A), and a representative example is shown in Fig. 4B. Two aspects of these results are striking when compared with results of previous assays for E2F-dependent *trans*-activation. First, the double-point mutant pm928/961, which alters the CR2 sequence and abolishes E2F-dependent activation, was not impaired for activation of the *hsp70* promoter. Second, the deletion of E1A N-terminal sequences, such as in the Δ2-36 and Δ15-35 mutants, which does not affect E2F-dependent activation, abolished the ability of the E1A$_{12S}$ protein to activate the *hsp70* promoter. Deletion of the N-terminal portion of CR1 (Δ38-67) reduced the capacity of E1A$_{12S}$ to *trans*-activate the *hsp70* promoter, as did the point mutant pmHN3 (Fig. 4A). In contrast, a point mutant with a change at amino acid residue 21 (pmDA21) was fully active for *hsp70* activation, as were deletions involving the C-terminal portion of CR1 (Δ51-116) and the sequence between CR1 and CR2 (Δ73-120) that were also fully functional for *hsp70* promoter activation.

The failure of the N-terminal mutants to activate the *hsp70* promoter was not likely due to a reduced stability of the mutant proteins, since these mutants were previously shown to activate E2F-dependent transcription (46). Moreover, as indicated by direct assays of proteins in transfected cells, the level of the E1A$_{12S}$ WT and Δ2-36 proteins was found to be equivalent (Fig. 4C). We therefore conclude that sequences at the N terminus of the E1A$_{12S}$ protein are essential for activation of the *hsp70* promoter, whereas sequences within CR2 are dispensable. Moreover, this specificity is distinctly different from that required for the E2F-dependent activation of transcription. We conclude that the E1A$_{12S}$ protein contains at least two distinct domains involved in the activation of transcription as a consequence of the targeting of distinct transcription factors.

DISCUSSION

A variety of studies have shown that three regions of the adenovirus E1A$_{12S}$ protein are essential for transforming activity (Fig. 5). These include sequences at the extreme N terminus of the protein, sequences within CR1, and sequences within CR2. Several observations have suggested that these three sequence elements constitute two func-

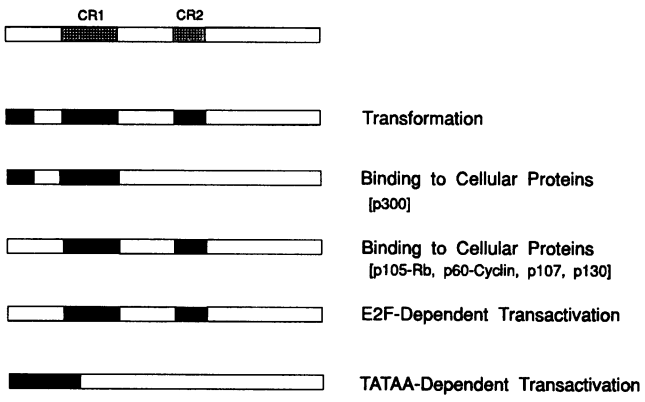

FIG. 5. Distinct *trans*-activation functions in separate domains within the $E1A_{12S}$ product. Depicted at the top is a schematic of the 243-aa $E1A_{12S}$ protein containing the CR1 and CR2 domains. The segments of E1A required for E2F-dependent *trans*-activation of the E2 promoter are depicted extending from residues 38 to 73 and 124 to 135 (46). These same regions generally correspond to those shown to bind to several cellular proteins, including the p105 retinoblastoma gene product, p60 cyclin A, p107, and p130 (11, 13, 59, 64), all of which except p130 are in association with E2F (2, 3, 6, 7, 10, 38). The segments of E1A required for *hsp70* promoter *trans*-activation are at the amino terminus extending to residue 50. This region overlaps with sequences that have been shown to bind to the cellular p300 (11, 59, 64). Definition of the segments required for E1A-dependent transformation in conjunction with the activated *ras* oncogene derives from various previous studies and includes residues 1 to 26, 35 to 77, and 121 to 139 (23, 30, 36, 59, 63, 64).

tional, albeit overlapping, domains. The N-terminal sequence, together with a portion of CR1, appears to contribute one functional domain, whereas the CR2 sequence, and again a portion of CR1, represents a distinct functional domain. For instance, N-terminal mutants and CR2 mutants are both defective for transformation but can complement one another in *trans* in transformation assays (35, 59). In addition, the sequences involved in the binding of E1A to a group of cellular proteins also define two distinct domains. The interaction of a 300-kDa protein with E1A requires extreme N-terminal sequence and part of CR1 (approximately aa 1 to 25 and 35 to 76) (11, 59, 64), whereas proteins of 130, 107, 105, (the retinoblastoma gene product), and 60 (cyclin A) kDa, interact with E1A dependent on the CR1 and CR2 sequences but independent of the extreme N terminus (13, 64). The results presented here suggest that activation of the *hsp70* promoter is a function of the N-terminal and CR1 domains, while previous results have shown that activation via the E2F factor is a function of the CR1 and CR2 functional domains. On the basis of these observations, we suggest that the two independent, functional domains within the $E1A_{12S}$ product act to target distinct transcription factors for the activation of transcription.

The activation of E2 transcription by the $E1A_{12S}$ product appears to involve the liberation of transcription factor E2F from complexes containing either the cyclin A protein (2, 10, 38) or the retinoblastoma protein (2, 6, 7, 18). It appears that the complexed E2F is transcriptionally inactive, at least for the E2 promoter, and thus the release of free E2F results in the production of active factor. By analogy, we suggest that activation of the *hsp70* promoter by the $E1A_{12S}$ product may involve a similar event, although not involving the E2F factor.

Since activation of the *hsp70* promoter was found to be dependent on N-terminal E1A sequence, an analogy to E2F activation would implicate proteins that interact with E1A N-terminal sequence in this control. The 300-kDa protein (p300) is the only protein known to bind to the E1A N terminus. It is thus attractive to suggest that p300 might affect TATA-dependent activation by E1A by forming a complex with the TATAA-dependent TATA-binding protein (TBP), possibly blocking the function of TBP. The dissociation of such a complex by the E1A N terminus would lead to activation of *hsp70*. It is, however, also true that recent experiments demonstrate a direct DNA-binding capacity for p300 that does not include a TATAA sequence (49). In this case, direct interaction of p300 with DNA would not preclude protein contacts with TBP and in fact might facilitate them. It is also possible that p300, if complexed to TBP, might recognize DNA sequence in the vicinity of the TATAA element, thus providing context-dependent sequence specificity to the TBP promoter interaction. Finally, although the genetic analysis of E1A-p300 interaction and E1A-mediated *trans*-activation of the *hsp70* promoter suggests that the two events do not precisely coincide (the function of the N-terminal mutants correlates closely with p300 binding, but the persistence of *trans*-activation activity in the Δ51-116 mutant is somewhat at odds with the lack of stable association between this mutant protein and p300 [59]), we do not believe that this finding rules out p300 as a target. For instance, the Δ51-116 deletion might reduce the ability of E1A to remain stably bound to p300 without impairing the ability of E1A to dissociate a TFIID-p300 complex. In short, on the basis of the E2F analogy, we believe that p300 remains a viable candidate as a target for the $E1A_{12S}$-mediated activation of the *hsp70* promoter.

Another potential candidate that might be considered, even though it has not been identified as an E1A-binding protein, is the recently described Dr activity, a factor that is known to inhibit the action of TBP (22). The precedent of E2F suggests that the E1A protein, dependent on N-terminal sequences, might dissociate a TBP-Dr complex, releasing the transcription factor TBP for action at the *hsp70* promoter. Although a final determination of the relationship of this activation event to the previously described activation involving E2F must await the demonstration of an appropriate complex, these experiments do identify the promoter target and thus the likely transcription factor targeted for the activation.

Although the results presented in Fig. 3 and those of previous experiments (55) provide a clear demonstration that the TATAA element of the *hsp70* promoter is required for E1A-dependent transcriptional activation, the results of other studies have not found such a specificity (61, 65). The basis for the discrepancy is not clear, but we presume that it must reflect differences in the assay systems. For instance, if a TATAA-specific factor was not rate limiting in the particular cell lines used in these other experiments, or at least was not the only rate-limiting factor, then one would indeed observe a difference in the requirement for specific factors. Although both studies used HeLa cells, it is clear that HeLa cell cultures can vary widely, dependent on laboratory growth conditions and thus selection pressures. We also note that in the studies of Taylor and Kingston (61), the E1A-mediated activation of the *hsp70* promoter containing the simian virus 40 TATTTAT element was significantly (sixfold) reduced in comparison with the wild-type sequence. In short, we do not wish to suggest that the TATAA element is the only element targeted by E1A; indeed, we argue just the opposite. Nevertheless, we do suggest that the

TATAA element can be a target and, under the conditions of our experiments, can be a significant contributor to *trans*-activation. In other circumstances, this element may be only one of several targets.

We also do not wish to imply that the TATAA sequence in the *hsp70* promoter is the sole basis for specificity of activation by the E1A$_{12S}$ protein. Indeed, there must be additional complexity, since the E3 promoter is not activated by the E1A$_{12S}$ protein even though it does contain the same TATAA sequence. We thus presume that the context of the TATAA sequence must contribute to the specificity. TBP is now recognized to be a much more general transcription factor than previously believed, clearly playing a broader role than simply facilitating transcription of TATA-containing polymerase II promoters. TBP has been shown to be important for transcription of TATA-less polymerase II promoters (45) as well as polymerase I (8) and polymerase III (33, 54) promoters. It appears that distinct DNA-binding specificities are conferred upon TBP by a diverse set of TBP-associated factors (TAFs) (53). It is thus evident that core sequence recognition by TBP and associated TAFs could vary greatly according to the unique set of TAFs utilized. Conceivably, one or more of these TAFs may confer specificity for a particular TATA sequence in a particular sequence context. E1A may interact with such a TAF directly or indirectly through another factor. In this way, the TATAA element in one promoter may act differently from the same TATAA element in another promoter, such as the difference we see between *hsp70* and the adenovirus E3 promoter.

The role of the adenovirus E1A CR3 sequence in the regulation of early viral transcription is well established. Without question, this activity is critical to the progression of a replicative cycle and is sufficient to activate the entire program of early viral transcription, at least in the cell lines normally used for these studies. Interestingly, recent evidence suggests that at least a part of this process of activation may involve the TATA element, given the observation that the E1A$_{13S}$ product can interact with the transcription factor TBP (19, 28). The experiments presented in this report, as well as previous experiments concerning the targeting of E2F, now demonstrate that the remaining functional elements of the E1A coding sequence are also involved in transcription control. Moreover, each of these elements appears to be capable of functioning independently, and the elements can complement each other in *trans*. Thus, these experiments suggest the possibility that the entire E1A coding capacity may well be devoted to transcriptional control.

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