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Genetics

The 289-amino acid E1A protein of adenovirus binds zinc in a region that is important for trans-activation

(atomic absorption spectrophotometry/chloramphenicol acetyltransferase assay/site-directed mutagenesis)

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ABSTRACT **The E1A gene of adenovirus type 5 encodes two major proteins of 289 and 243 amino acid residues, which are identical except that the larger protein has an internal stretch of 46 amino acids required for efficient trans-activation of early viral promoters. This domain contains a consensus zinc finger motif (Cys-Xaa$_2$-Cys-Xaa$_{13}$-Cys-Xaa$_2$-Cys) in which the cysteine residues serve as postulated ligands. Atomic absorption spectrophotometry applied to bacterially expressed E1A proteins revealed that the 289-amino acid protein binds one zinc ion, whereas the 243-amino acid protein binds no zinc. Replacing individual cysteine residues of the finger with other amino acids destroyed the trans-activating ability of the 289-amino acid protein, even when structurally or functionally conserved amino acids were substituted. These results strongly suggest that the zinc finger of the 46-amino acid domain is intimately linked to the ability of the large E1A protein to stimulate transcription of E1A-inducible promoters. Furthermore, zinc binding to one of the mutant finger proteins suggests either that only a precise finger structure formed by the tetrahedral coordination of zinc to the four consensus ligands is required for trans-activation or, possibly, that one of several neighboring histidine residues in various combinations with three of the consensus cysteine residues normally coordinates zinc. How the zinc finger in E1A might interact with DNA or protein to bring about trans-activation is discussed.**

The E1A gene of adenovirus functions normally by transcriptionally activating (trans-activating) other early viral promoters, which leads to productive infection (1, 2). E1A gene products can also trans-activate the promoters of some cellular genes—e.g., heat shock and β-tubulin (3, 4)—and, conversely, can repress enhancer-stimulated transcription from the promoters of certain other genes—e.g., immunoglobulin and simian virus 40 early promoters (5–7). How these modes of positive and negative regulation by E1A may be involved in E1A-dependent viral transformation and tumorigenesis (reviewed in ref. 8) is unknown.

There has been an intense effort to understand how trans-acting E1A products stimulate cis-acting elements of inducible promoters. E1A transcripts of 12S and 13S differ only by the amount of their intervening sequences removed by splicing (9, 10). These mRNAs encode phosphoproteins of 289 and 243 amino acids (aa), which are identical except that the large protein, produced from the 13S mRNA, contains an additional internal stretch of 46 aa. The 243-aa protein trans-activates much less efficiently than the 289-aa protein when synthesized by viruses expressing one protein or the other (11, 12); interestingly, this disparity is not as great when the analysis involves microinjection of bacterial E1A proteins (13). Mutational analysis has shown that the 46-aa coding domain is required for the efficient trans-activation observed with the 289-aa protein (14–16). Furthermore, microinjection of a synthetic peptide containing this 46-aa domain has been reported to stimulate an early viral promoter (17). It remains unclear how the 289-aa protein stimulates its target promoters since a common inducible DNA response element has not been identified (18–23). However, various studies strongly indicate that the mechanism of E1A trans-activation involves recruitment of one or more cellular transcription factors (24–26).

The E1A coding region of adenovirus (Ad) contains a putative metal-binding sequence that resides within the 46-aa trans-activating domain of the large 289-aa protein but is not present in the small 243-aa protein. This consensus sequence is conserved in the large E1A protein of the different Ad subgroups, A, B, C, and E (27) (Fig. 1). Many viral and cellular proteins that bind DNA and/or regulate transcription contain potential metal-binding domains that have the consensus sequences Cys-Xaa$_{2-4}$-Cys-Xaa$_{2-15}$-(Cys or His)-Xaa$_{2-4}$-(Cys or His) or (Cys or His)-Xaa$_{2-4}$-(Cys or His)-Xaa$_{2-15}$-Cys-Xaa$_{2-4}$-Cys (28). The Cys, or Cys and His, residues are thought to form a tetrahedral complex with a metal ion such that the central Xaa$_{2-15}$ residues form a loop or finger structure that may interact with DNA (29). The prediction that some proteins containing this sequence bind zinc (28) was based on the observation that *Xenopus* transcription factor IIIA (TFIIIA), which was identified as a Zn metalloprotein (30), binds 7–11 mol of zinc and contains nine of these fingers (29, 31). More recently, purified transcription factor SP1, which contains three zinc fingers, was shown to lose DNA binding activity when incubated with the metal chelator EDTA (32). There is genetic evidence suggesting that finger sequences of the yeast trans-activating proteins ADR1 (33) and GAL4 (34) are essential for function. Although finger motifs are found in many proteins that are known or thought to bind DNA (e.g., steroid receptors, the large T antigens of papovaviruses, and proteins encoded by *Drosophila* segmentation genes), these structures could also serve in protein interactions (reviewed in refs. 28 and 35). Here, we use the term finger to denote a structure without implying a specific function.

In this study, we show that the Ad type 5 (Ad5) 289-aa E1A protein contains a single zinc binding domain within the 46-aa region. This protein binds one zinc ion (Zn^{2+}) in contrast to the smaller 243-aa E1A protein, which binds no zinc and does not contain the 46-aa region. Site-directed mutagenesis of the individual Cys residues of the E1A finger rendered the 289-aa

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Abbreviations: aa, amino acid(s); TFIIIA, transcription factor IIIA; Ad, adenovirus; Ad5, etc., Ad type 5, etc.; wt, wild type.

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Ad12	Subgroup A	G	H	N	C	K	S	C	E	H	H	R	N	S	I	G	N	T	D	L	M	C	S	L	C	Y	L	R
Ad7	Subgroup B	G	H	G	C	K	S	C	E	F	H	R	N	N	I	G	M	K	E	L	L	C	S	L	C	Y	M	R
Ad5	Subgroup C	G	H	G	C	R	S	C	H	Y	H	R	R	N	I	G	D	P	D	I	M	C	S	L	C	Y	M	R
Ad4	Subgroup E	G	H	G	C	K	S	C	E	F	H	R	I	N	I	G	D	K	A	V	L	C	A	L	C	Y	M	R

FIG. 1. Amino acid sequence comparison (single-letter code) of the E1A finger domains of the different serotypes of Ad. The four consensus Cys ligands for binding zinc are indicated by the Cs with the asterisks. Conserved amino acid residues are underlined. Serotypes A, B, C, and E are represented by Ad12, -7, -5, and -4, respectively. The complete E1A coding regions of these viruses are compared in ref. 27.

protein incapable of trans-activation. Zinc binding to one of the mutant proteins suggests that only a precise finger structure formed by the tetrahedral coordination of zinc to the four consensus Cys ligands is required for trans-activation or that zinc normally coordinates with various combinations of three of the consensus Cys residues and one of several neighboring His residues. Ways in which a zinc binding domain could participate in the ability of E1A to stimulate transcription of other promoters are discussed.

MATERIALS AND METHODS

Purification of Bacterial E1A Proteins. Plasmid vectors pAS1-E1A410 and pAS1-E1A412 were used to express the E1A 289- and 243-aa proteins, respectively, in *Escherichia coli* AR120 as described (13, 36) with few modifications. Five hours after induction with nalidixic acid, cells were pelleted, resuspended in buffer A [50 mM Tris·HCl, pH 8.0/1 mM EDTA/1 mM dithiothreitol/1 mM phenylmethylsulfonyl fluoride/5% (vol/vol) glycerol] containing lysozyme (1 mg/ml), incubated for 30 min on ice, and sonicated. The insoluble material containing E1A protein was pelleted, resuspended in buffer A, sonicated, and washed twice in buffer A. To solubilize E1A, the final pellet was resuspended in buffer A containing 6 M urea; insoluble debris was pelleted. The supernatant was applied to a DEAE-Trisacryl column (1.5 × 5 cm) (IBF Biotechnics) equilibrated in 50 mM Tris·HCl, pH 8.0/50 mM NaCl/1 mM dithiothreitol/2 M urea/0.5 mM EDTA, and the column was washed in the same buffer. Protein was eluted by a linear NaCl gradient (0.05–1.0 M) and fractions were examined by Coomassie blue-stained $NaDodSO_4$/polyacrylamide gels. E1A protein fractions of highest purity were used for zinc binding analysis.

Atomic Absorption Spectrophotometry. Purified E1A proteins (150 μg) were dialyzed against phosphate-buffered saline (PBS) (10 mM sodium phosphate, pH 7.2/140 mM NaCl/5 mM KCl)/5% glycerol/0.1 mM dithiothreitol/0.1 mM $ZnCl_2$ for 20 hr at 4°C. Dialysis was continued against 10 mM Chelax-100-treated phosphate buffer, pH 7.3/0.2 M NaCl/5% glycerol/0.1 mM dithiothreitol, and varied concentrations of EDTA for >20 hr with one or two changes of buffer. Millipore Super Q water was used in all solutions. The zinc content was measured with a Perkin–Elmer model 4000 atomic absorption spectrophotometer equipped with a HGA 400 graphite furnace as described (37). Zinc content was calculated from a standard curve with a zinc standard solution (Fisher). All labware that came in contact with the protein samples was pretreated with 30% nitric acid or 10 mM EDTA. Protein concentrations were determined routinely by the Bio-Rad assay (38) and checked for accuracy by amino acid analysis.

Site-Directed Mutagenesis. The *Sma* I/*Xba* I fragment of genomic Ad5 E1A (base pairs 1007–1339) or E1A 13S cDNA were cloned into the polylinker sites of M13 and oligonucleotides (13- or 18-mer) were used to direct mutagenesis by the method of Kunkel (39). Mutations were verified by DNA sequencing (40). For analysis of mutant E1A proteins, the altered *Sma* I/*Xba* I fragments derived from genomic DNA were cloned into pEKpm975 (15) for trans-activation studies and the mutated 13S cDNA fragment was cloned into pAS1-E1A410 (36) for expression in bacteria.

Trans-Activation Assay. HeLa cells were cotransfected with E1A plasmids and the reporter plasmid p3CAT as described (16, 41–43). The p3CAT plasmid contains the chloramphenicol acetyltransferase gene driven by the E3 promoter of adenovirus (43). Cells were harvested after 40 hr and extracts were assayed for chloramphenicol acetyltransferase activity as described (16, 43).

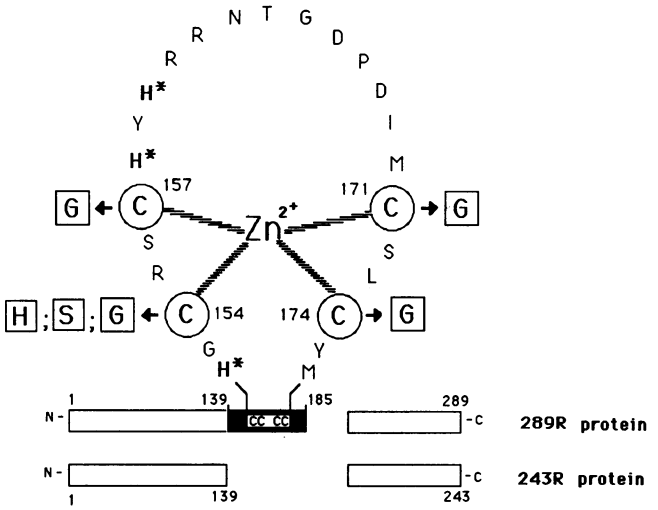
RESULTS

Zinc Binds to Only the Large Protein of E1A. The 289- and 243-aa proteins encoded by the E1A gene of Ad5 are identical except that the large protein contains an internal stretch of an additional 46 aa (10). As noted by Berg (28), a consensus zinc finger motif ($Cys\text{-}Xaa_2\text{-}Cys\text{-}Xaa_{13}\text{-}Cys\text{-}Xaa_2\text{-}Cys$) resides within the 46-aa domain of the 289-aa protein but is not present in the 243-aa protein (Fig. 2). We predicted that if the consensus sequence actually does serve as a metal coordination site, then the 289-aa protein should bind zinc but the 243-aa protein should not.

The 289- and 243-aa E1A proteins were expressed in *E. coli*, extracted from the cell lysate, and purified in a single chromatographic step. Both bacterial E1A proteins purified in this way trans-activate when microinjected into mammalian cells, although the larger protein is more efficient (44). Both the 289- and 243-aa bacterial E1A proteins were purified to near homogeneity, as shown in Fig. 3. Before analysis of zinc content, the purified E1A proteins were dialyzed in buffer containing 0.1 mM $ZnCl_2$ to restore metal that may have been removed during purification. The proteins were then exhaustively dialyzed in buffer devoid of zinc to remove unbound or loosely bound metal.

Binding of zinc to the E1A proteins was tested with an atomic absorption spectrophotometer equipped with a graphite furnace to enhance the sensitivity of the measurements. By this analysis, ≈1 mol of zinc bound to 1 mol of the 289-aa protein, whereas little if any zinc bound to the 243-aa protein (Table 1). The ability of only the larger E1A protein to complex with zinc correlated with the presence of the consensus finger motif located within its unique 46-aa domain. Zinc bound to the 289-aa protein was also assayed after dialysis in the presence of the metal chelator EDTA. As shown in Table 1, ≈50% of the zinc bound to the 289-aa protein was lost upon treatment with 0.1 mM EDTA (Table 1). All zinc was removed from the 289-aa protein when dialyzed in 1.0 mM EDTA.

Cys Residues of the Finger Are Required for Trans-activation. All four Cys residues predicted to form a tetrahedral complex with zinc, thereby generating a peptide finger, are located within the 46-aa domain of the 289-aa protein that is required for efficient trans-activation (14–16). Each residue was substituted with one or more amino acids by site-directed mutagenesis to assess whether the individual Cys residues are required for trans-activation and, by inference, whether an appropriate finger structure may be necessary for trans-activation. Cys-154, -157, -171, and -174 were individually substituted with Gly and in the case of Cys-154,



mutant name	nucleotide change	(codon) and change	amino acid change
Gly-154	1019 T to G	(154) TGC to GGC	Cys to Gly
Ser-154	1019 T to A	(154) TGC to AGC	Cys to Ser
His-154	1019 T to C; 1020 G to A	(154) TGC to CAC	Cys to His
Gly-157	1028 T to G	(157) TGT to GGT	Cys to Gly
Gly-171	1070 T to G	(171) TGT to GGT	Cys to Gly
Gly-174	1079 T to G	(174) TGC to GGC	Cys to Gly

FIG. 2. (*Upper*) Amino acid sequence (single-letter code) of the zinc finger within the 46-aa domain of the 289-aa E1A protein of Ad5. The consensus finger contains four Cys residues at positions 154, 157, 171, and 174, shown here coordinated to a single zinc ion (Zn^{2+}). The 243-aa protein is identical to the 289-aa protein except that it does not contain the 46-aa domain (residues 139–185; solid area). His residues designated by the asterisks (H*) could possibly act in place of Cys-154 or Cys-157 to form alternative fingers by coordinating with the remaining three Cys residues (see text); His-152 and His-160 are conserved in the E1A genes of other serotypes, whereas His-158 is not. Six mutant E1A 289-aa proteins containing single amino acid substitutions of the Cys residues (boxed) are shown. (*Lower*) The substitutions are described.

with Ser and His as well (Fig. 2). The Ser substitution represents the most conserved structural change, whereas the His substitution represents the most conserved functional change, as zinc can bind to either Cys or His. All of these mutations were produced in a plasmid that expresses only the 289-aa E1A protein (15) and tested in HeLa cells for their ability to trans-activate the early region 3 (E3) promoter of Ad. The assay involved cotransfection of the E1A plasmids with a reporter plasmid containing the chloramphenicol acetyltransferase gene driven by the E3 promoter (45). Forty hours after transfection, cell extracts were prepared and assayed for the ability to acetylate [^{14}C]chloramphenicol. As expected, the E3 promoter was efficiently activated in the

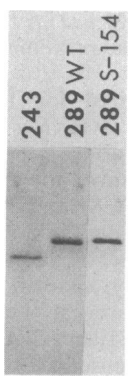


FIG. 3. E1A proteins purified from *E. coli*. *E. coli* containing plasmids pAS1-E1A412, pAS1-E1A410, and pAS1-E1A410-Ser were induced to express the wt 243-, wt 289-, and the Ser-154 mutant 289-aa proteins, respectively. E1A proteins were extracted from cell lysates with urea, purified by anion-exchange chromatography, electrophoresed on a 10% $NaDodSO_4$/polyacrylamide gel, and stained with Coomassie brilliant blue.

Table 1. Zinc content of the 289- and 243-aa proteins by atomic absorption spectrophotometry

E1A protein	Zinc content, mol per mol of protein: EDTA, 0 mM	EDTA, 0.1 mM	EDTA, 5 mM
289 aa	0.96	0.50	0.00
289 aa (Ser-154)	1.04	0.41	0.15
243 aa	0.10	—	—

The E1A proteins purified from bacteria were dialyzed in the Chelax-100-treated buffer containing $ZnCl_2$ and then against zinc-free buffer either without or with EDTA. Zinc content was measured by atomic absorption spectrophotometry. Values are representative of several determinations obtained from different E1A protein preparations. The accuracy for all determinations was ±0.1.

presence of the wild-type (wt) 289-aa E1A plasmid (Fig. 4). However, chloramphenicol acetyltransferase activity was not obtained with any of the mutated E1A plasmids (Fig. 4). Immunoblot analysis confirmed that the mutant and wt E1A proteins were synthesized at similar levels in the transfected HeLa cells (data not shown). These results signify that trans-activation by the 289-aa E1A protein requires all four Cys residues, which is consistent with their potential to bind zinc resulting in the formation of a specific finger structure.

Zinc Binding Ability of a 289-aa E1A Protein Containing a Cys to Ser Substitution. We wished to know whether a mutant 289-aa protein, rendered defective for trans-activation by substitution of one Cys residue of the finger, would be defective for zinc binding as well. A 289-aa E1A protein in which Ser was substituted for Cys-154 was synthesized in bacteria, purified, and analyzed by atomic absorption spectrophotometry. As shown in Table 1, the Ser-154 mutant protein bound the same amount of Zn^{2+} as the wt 289-aa protein—i.e., 1.0 mol of Zn^{2+} per mol of protein. Furthermore, dialysis in the presence of EDTA indicated that Zn^{2+} appears to bind as strongly to the Ser-154 mutant protein as it does to the wt 289-aa protein. Possible explanations for this surprising finding are discussed below.

DISCUSSION

We have shown that 1 mol of the 289-aa E1A protein of Ad can bind 1 mol of Zn^{2+}, which correlates with the presence

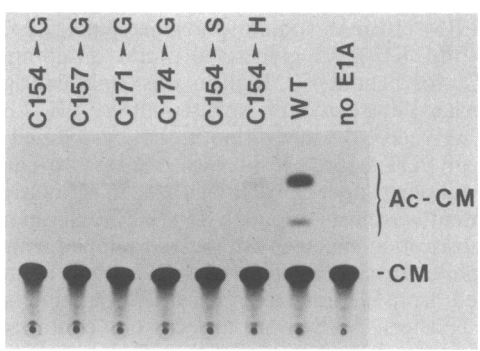


FIG. 4. Assay for trans-activation of the E3 promoter by E1A finger mutants. HeLa cells were cotransfected with 3.6 μg of the reporter plasmid pE3CAT and 3.6 μg of the mutant E1A plasmids or the wt E1A plasmid. Each of the E1A plasmids expresses the 289-aa protein only. After 40 hr, cells were harvested and the cell extracts were incubated with [^{14}C]chloramphenicol (CM). The acetylated forms of [^{14}C]chloramphenicol (Ac-CM) were separated by TLC and XAR-5 film was exposed to the TLC plate. Lane designations indicate pE3CAT plus the mutated E1A plasmids as follows: Gly-154, Gly-157, Gly-171, Gly-174, Ser-154, His-154, wt E1A plasmid pEKpm975, 3.6 μg of carrier plasmid in the absence of E1A. G, S, and H, glycine, serine, and histidine, respectively.

of a single consensus zinc finger (Cys-Xaa$_2$-Cys-Xaa$_{13}$-Cys-Xaa$_2$-Cys) located within the 46-aa internal domain. The strength of this finding is enhanced by the fact that essentially no zinc bound to the 243-aa E1A protein, which lacks the 46-aa domain but otherwise has an amino acid sequence that is identical to the 289-aa protein. Moreover, zinc bound to the 289-aa protein could be removed by EDTA. Sensitivity to metal chelation was also recently demonstrated for SP1, in which EDTA was able to eliminate the binding of this finger protein to its specific DNA recognition sequence (32).

The central question arising from this study is whether the metal finger of the 289-aa protein functions in trans-activation. When any of the four Cys residues of the finger that are thought to complex with Zn^{2+} were substituted with Gly, the trans-activating function of the 289-aa E1A protein was completely lost. Moreover, whether structurally (Cys to Ser) or functionally (Cys to His) conserved substitutions were made at position 154, the trans-activating function of E1A was also lost. Since His can also coordinate with Zn^{2+}, this indicates that there is a stringent requirement for Cys at position 154; the effect of similar substitutions of the other three Cys residues of the finger has yet to be examined. It should be noted that trans-activation by the human estrogen receptor protein did not occur when a pair of His residues was substituted for one of the normal pairs of Cys residues in its putative finger (46) and that a Ser in place of Cys within one of the putative finger domains of the Krüppel gene product of *Drosophila* correlated with loss of specific biological activity (47). Trans-activation by the yeast protein ADR1 was prevented if Cys residues in either of its two putative fingers were substituted by nonconserved amino acids (33). Interestingly, two previously characterized E1A mutants, hr3 and hr4, contain amino acid substitutions within or adjacent to the finger and fail to trans-activate (16) as does an Ad12 E1A mutant containing a 4-aa insertion in the middle of the finger (R. Vasavada and R.P.R., unpublished data). In light of the data, we suggest that these 289-aa mutant proteins are defective for trans-activation because they fail to form appropriate finger structures.

Surprisingly, the 289-aa bacterially expressed protein containing the Cys to Ser substitution at position 154 was not diminished in its capacity to bind Zn^{2+}. It also appears that the wt and mutant E1A proteins have similar affinities for zinc since they were nearly identical in sensitivity to chelation by EDTA. Retention of zinc binding by the mutant may be due to the formation of a metal complex by using a combination of sulfhydryls from the three remaining Cys residues and (*i*) some other amino acid within the E1A protein—e.g., a thiol or imidazole group from another Cys or His, respectively, (*ii*) the hydroxyl group from Ser-154, or (*iii*) a water molecule. Predictably, such substituted ligands would affect the conformation of the finger so that trans-activation is prevented. Recently, it was suggested that participation of ligands other than thiols could account for the finding that truncated yeast metallothionein proteins, missing several of the normal metal binding cysteines, were still capable of binding copper (48).

Also, it is very likely that zinc binding to the E1A Ser-154 mutant protein can be accounted for by any one of several other potential metal binding sites that closely resemble the consensus finger sequence (28). These alternative sequences differ only from the more probable one (28) presented here (Cys-154, Cys-157, Cys-171, Cys-174) by using in place of Cys-154 the conserved residues His-152 to generate (His-152, Cys-157, Cys-171, Cys-174) and His-160 to generate (Cys-157, His-160, Cys-171, Cys-174) or in place of Cys-157, the nonconserved His-158 residue to generate (Cys-154, His-158, Cys-171, Cys-174) (see Fig. 2). The similar sensitivity to metal chelation seen for both the wt 289-aa and E1A Ser-154 mutant proteins is in accord with the comparable zinc binding predicted for both the consensus and alternative metal binding sequences. Although these alternative sequences could also form the true finger of the wt 289-aa protein, this is thought not to be the case since the position at which His occurs in the order of ligands (His-Cys-Cys-Cys or Cys-His-Cys-Cys) has not been observed in the large number of known and potential eukaryotic finger proteins (28); furthermore, Cys binds to zinc more tightly than does His (49). From these results, we suggest that a precisely formed finger created by the tetrahedral coordination of zinc to the consensus Cys ligands appears to be required for trans-activation. However, unequivocal evidence that the coordination site maps to these four Cys residues awaits analysis by physical methods, such as extended x-ray absorption fine structure analysis, as was done for TFIIIA (50). Regardless of which of these ligands is used to coordinate zinc in the E1A Ser-154 mutant, our results reveal that zinc binding to the 289-aa protein alone is not sufficient for trans-activation. It is important to note that these results represent the first quantitative analysis of binding of Zn^{2+} to a finger in which one of the putative ligands has been altered.

How could the finger within the trans-activating domain of the 289-aa protein participate in the process of trans-activation? One possibility is that the E1A finger serves to contact critical elements on the promoter region of DNA. Indeed, the multiple finger proteins TFIIIA and SP1 have been shown to require zinc for DNA binding (30, 32), and there is genetic evidence suggesting that this may be so for the single finger protein GAL4 (34). However, E1A protein alone does not appear to bind DNA (51), and there is no obvious target element common to all E1A-inducible promoters (18–23). On the other hand, bacterially produced E1A protein can exhibit DNA binding when mixed with cellular extracts (51, 52), and there is compelling evidence that trans-activation by E1A occurs through an interaction with cellular transcription factors (24–26, 53). In the presence of E1A, there is enhanced binding of a cellular factor upstream of the E2 promoter (24), and mutational analysis of the E1B promoter suggests that E1A increases the activity of the "TATA" box transcription factor (25). In addition, E1A appears to mediate stimulation of RNA polymerase III promoters by increasing the active concentration of TFIIIC (54, 55). Also, the nonfunctional 289-aa protein of the E1A missense mutant hr5 is able to inhibit the wt 289-aa protein from trans-activating other viral promoters by apparently competing for a transcription factor (26). Finally, cellular proteins have been shown to copurify with E1A (56, 57) and, recently, bacterially produced E1A has been shown to bind directly to specific DNA binding proteins that interact with early viral promoters (X-P. Shi, R. Weinmann, and M.R., unpublished data). Thus, it is quite conceivable that E1A interacts with one or more cellular factors to generate active transcription complexes on the DNA. Given the wide range of E1A-inducible promoters, one might speculate that the 289-aa protein associates with transcription factors such that the E1A finger contacts the DNA and in so doing promotes the formation or stabilization of a transcription complex on the promoter. In this model, cellular transcription factors could dictate sequence-specific binding to the DNA and the 289-aa protein could act to increase the half-life of the transcription factor complex, which would result in stimulated transcription. Such a mechanism would not preclude the ability of E1A to modify factors with which it interacts. This model would also be compatible with E1A interacting with a preformed transcription factor complex on the promoter DNA or with factors prior to the formation of the transcription complex. Alternatively, the E1A finger may not function in DNA binding, but it might serve as a structure needed to bind to a factor for stabilization of a complex or perhaps modifying it in such a way as to increase transcriptional activity.

Given the fact that the Cys residues of the finger are conserved in the large E1A proteins of the different serotypes of E1A, and that a specific structure of the finger may be essential for trans-activation, it is intriguing to consider whether other conserved residues participate in the formation of this structure or make critical contacts with DNA or another protein (Fig. 1). Despite the substantial sequence divergence in the fingers of the different serotypes, it is not unreasonable to predict that the three-dimensional structures of these fingers are conserved since there appears to be a common trans-activating mechanism. In this regard, E1A may provide an excellent model for the study of protein evolution with respect to structure–function relationships.

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