The Viral Transactivator E1A Regulates the Mouse Mammary Tumor Virus Promoter in an Isoform- and Chromatin-specific Manner*

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Proteins encoded by the adenovirus E1A gene regulate both cellular and viral genes to mediate effects on cell cycle, differentiation, and cell growth control. We have identified the mouse mammary tumor virus (MMTV) promoter as a target of E1A action and investigated the role nucleo-protein structure plays in its response to E1A. Both 12 and 13 S forms target the MMTV promoter when it has a disorganized and accessible chromatin configuration. However, whereas the 13 S form is stimulatory, the 12 S form is repressive. When the MMTV promoter adopts an organized and repressed chromatin structure, it is targeted only by the 13 S form, which stimulates it. Although evidence indicates that E1A interacts with the SWI/SNF remodeling complex, E1A had no effect on chromatin remodeling at the MMTV promoter in organized chromatin. Analysis of E1A mutants showed that stimulation of the MMTV promoter is mediated solely through conserved region 3 (CR3), included in the 13 S but not the 12 S protein. In addition to CR3, the E1A proteins contain two other domains, CR1 and CR2, which are conserved among the various adenovirus species. These three domains are necessary for most of the cellular effects of E1A expression (2). CR1 and CR2 are important for mediating effects on cell cycle and ultimately cell transformation. They have been shown to interact with transcriptional cofactors CBP/p300 (3), PCAF (4), and the tumor suppressor Rb and its family members (5). CR1 and the extreme N terminus of E1A have also been shown to be necessary for a functional interaction with the yeast SWI/SNF complex (6, 7), which catalyzes ATP-dependent chromatin remodeling necessary for transcriptional regulation at some gene promoters (8). Thus, through interaction with both histone acetyltransferases, such as p300, and ATP-dependent nucleosome remodeling complexes, such as SWI/SNF, E1A may play an important role in alteration of chromatin structure.

The study of viral transactivators such as adenovirus E1A, herpes VP16, and SV40 T antigen has provided valuable information about cell cycle regulation and transcription (1). The E1A proteins of adenovirus mediate transcriptional regulation of both viral and cellular genes. These changes in transcription facilitate the viral life cycle, induce cell cycle progression, and can sometimes lead to cellular transformation. The mechanisms by which E1A causes transcriptional modulation are both direct and indirect and have been the focus of a multitude of studies.

The E1A gene is expressed as a family of proteins through alternative splicing. The most predominant protein species are the 13 and 12 S variants, which differ by a domain, referred to as conserved region 3 (CR3), included in the 13 S but not the 12 S protein. In addition to CR3, the E1A proteins contain two other domains, CR1 and CR2, which are conserved among the various adenovirus species. These three domains are necessary for most of the cellular effects of E1A expression (2). CR1 and CR2 are important for mediating effects on cell cycle and ultimately cell transformation. They have been shown to interact with transcriptional cofactors CBP/p300 (3), PCAF (4), and the tumor suppressor Rb and its family members (5). CR1 and the extreme N terminus of E1A have also been shown to be necessary for a functional interaction with the yeast SWI/SNF complex (6, 7), which catalyzes ATP-dependent chromatin remodeling necessary for transcriptional regulation at some gene promoters (8). Thus, through interaction with both histone acetyltransferases, such as p300, and ATP-dependent nucleosome remodeling complexes, such as SWI/SNF, E1A may play an important role in alteration of chromatin structure.

The CR3 region of 13 S E1A is necessary for transactivation of other adenovirus gene promoters and some cellular promoters (9). It interacts with various transcription factors as well as components of the TFIIID complex required for RNA polymerase II-mediated transcription, such as TBP (10–12) and several of the TBP-associated factors (TAFs) (13–15). More recently, it was shown to interact with human Srb-Mediator complex through a protein referred to as hSUR2 (13, 16). The CR3 region is thought to stimulate the basal transcription machinery, although the precise mechanism is not clear. However, E1A activates only a subset of genes, so it has been proposed that targeting to specific gene promoters is achieved through interaction with various transcription factors, such as ATF-2 (reviewed in Refs. 1 and 2). The role of the other conserved domains in facilitating the function of the CR3 region is not clear in many cases. It is certainly possible that transcriptional coactivators CBP/p300 and PCAF, which interact with CR1, may play a supportive role in the stimulation of the basal transcription machinery by CR3. CBP/p300 interaction is important in mediating 12 S E1A stimulation of the peripheral cell nuclear antigen promoter (17).

The mouse mammary tumor virus (MMTV) promoter is activated by glucocorticoids. The activated steroid receptors are known to interact with factors such as CBP/p300 and PCAF, which play an important role in transactivation of target pro-

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1 The abbreviations used are: CR, conserved region; PEPCK, phosphoenolpyruvate carboxykinase; MMTV, mouse mammary tumor virus; GR, glucocorticoid receptor; CBP, CREB-binding protein; LTR, long terminal repeat; GFP, green fluorescent protein; MACS, magnetic affinity cell sorting; Dex, dexamethasone; PBS, phosphate-buffered saline; IL2R, interleukin-2 receptor; PCAF, p300/CBP-associated factor.
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EXPERIMENTAL PROCEDURES

Construction of E1A Expression Vectors—Plasmids pE1A-WT and p12S E1A, expressing both 12 S and 13 S isoforms and just the 12 S isoform, respectively, were kindly provided by Dr. Elizabeth Moran (Temple University). Plasmids containing cDNA sequences for 13 S, 13Sα140–146, 13Sα169–174, and 13Sα180–188 were obtained from Dr. Robert Ricciardi (University of Pennsylvania) and have been described (14). The 13 S E1A expression vector was constructed by replacing an Xmal/Blpl fragment containing CR3 as well as the intron between CR2 and CR3 in the wild type E1A expression vector with the same fragment from the 13 S E1A cDNA. The resulting construct, p13S E1A, does not contain the intron and expresses only the 13 S E1A isoform. E1A mutants RG2 and αRb were derived from plasmids p125-RG2 and p125-YH47/928 (kind gifts from Dr. Elizabeth Moran) containing previously described point mutations in the binding sites for CBP/p300 and PCAF1, respectively (23). A XI fragment containing the Rb binding motif was removed from the p13S vector and used to replace the analogous fragment in pE1A-WT to generate the ΔRb mutant. The RG2 mutant was generated by replacing an EcoRI/Clal fragment in pE1A-WT with the analogous fragment from the p125-RG2 plasmid. The PCAF binding site mutants were created through site-directed mutagenesis using pE1A-WT and the following two sets of oligonucleotides: 5'-GACGGCGCGCAAGAACGAGGAGG-3' and 5'-CTCTCGTTGGATTTGGGCGCTC-3' (ΔPCAF1), 5'-GCGGGCCTTCACGCGGAGGATGGTTCT-3' and 5'-CGAAAACGCTTCGCGTTGAGTCGCGGCGGCGG-3' (ΔPCAF2). ΔPCAF1 carries two amino acid point mutations, E55K and D56N (24), whereas ΔPCAF2 carries three, E55A, D56A, and E59A (4). The transcription adaptor motif mutant carries point mutations in CR1 (F66A, D68A, V70A, and L72A), in a region shown to interact with the transcription adaptor motif of CBP (30 cycles) using Taq polymerase and a 32P-labeled primer containing sequences from the transcription start site in the MMTV promoter. Amplified products were separated on 8% denaturing gels, which were dried and exposed to phosphorimaging screens.

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RESULTS

E1A Modulates Transcription at Structurally Distinct MMTV Templates—The effect of E1A expression was tested on MMTV reporter constructs that were either transiently transfected (transient MMTV template) or incorporated into cellular chromatin (stable MMTV template) using a mouse mammary adenocarcinoma cell line, 1470.2. To examine E1A effects on an endogenous template in the absence of an I-2R-encoding reporter, 1470.2 cells were transfected with an E1A vector expressing both 12 S and 13 S proteins along with an expression vector for the Tac subunit of the interleukin-2 receptor (IL2R). The IL2R is inserted into the cell membrane and allows for purification of transfected cells via magnetic affinity cell sorting using beads coated with an IL2R antibody. Cell sorting is required for accumulation of bovine papilloma virus sequences. Cell line 3617 was derived from 3134 cells as previously described (26). They express GR tagged with green fluorescent protein (GFP) and contain 200 tandemly integrated copies of a transcription unit in which the full-length MMTV LTR drives the expression of the v-ras gene. Cells were maintained in Dulbecco’s modified Eagle’s medium containing 10% fetal bovine serum. Transfections were carried out by electroporation in a Squareporator (BTX, Genetronics) at 160 V, 40 mA, and four pulses. The amount of E1A expression vector transfected varied and was based on obtaining similar levels of expression for all of the isoforms and mutants as determined by Western blotting of extracts from transfected cells. For experiments in which β-actin was analyzed, an expression vector for transcription of the subunit of the interleukin-2 receptor was included in transfections. This protein was used as a tag for magnetic affinity cell sorting (MACS) as previously described (27). For experiments in which the activity of a transiently transfected MMTV reporter construct was to be measured, transfections included 5–10 μg of pLTRluc, which contains the full-length MMTV LTR driving transcription of the luciferase gene.

Luciferase Assays and RNA Analysis—For luciferase assays, transfected cells were plated in six-well dishes and allowed to recover overnight prior to treatment. Cells were treated with or without 100 nM Dex for 6 h prior to harvest. Preparation of extracts and luciferase assays have been previously described (28). For analysis of RNA, transfected cells were plated in 150-mm dishes. After overnight recovery, the cells were treated with or without 100 nM Dex for 3 h prior to harvest and MACS. RNA was isolated from beaded cell pellets as described previously (27). Analysis of MMTV RNA levels was carried out using S1 nuclease assay as previously described (27). Levels of β-actin mRNA were also determined for normalization purposes.

Nuclei Digestion and Chromatin Analysis—Transfected cells were seeded in 150-mm plates. After overnight recovery, they were treated with or without 100 nM Dex for 1 h prior to harvest and MACS. Nuclei were isolated from transfected cell populations as previously described (27). For analysis of Snc1 access, nuclei were digested with Snc1 (10 units/μg of DNA) for 15 min at 30 °C. DNA was then processed and purified as previously described and digested to completion with DpnII. For analysis of NF1 binding, nuclei were digested with HaeIII (5 units/μg of DNA) and a promoter (1 unit/μg of DNA) for 15 min at 37 °C. DNA was processed and purified as previously described (27). Digestion products were linearly amplified (30 cycles) using Taq polymerase and a 32P-labeled primer containing sequences from the transcription start site in the MMTV promoter. Amplified products were separated on 8% denaturing gels, which were dried and exposed to phosphorimaging screens.

Cell line 1470.2 was derived from plasmids containing the MMTV sequences of interest and is activated by the glucocorticoid receptor (GR) (22). In the context of cell line 1470.2, it attains a highly organized chromatin structure and is activated by GR make it a potential target for E1A action. Our study aimed to determine whether E1A affected MMTV promoter activity in either structural context and, if so, through which E1A domain and potential set of interacting factors did it mediate those effects.

We found that E1A proteins target the MMTV promoter in both structural contexts but do so differentially. While the 12 S protein repressed the transiently transfected MMTV template, it had no effect on the template in organized chromatin (referred to as the stable MMTV template). In contrast, the 13 S protein significantly stimulated transcription from both MMTV templates. Further investigation of the stimulatory effect of the 13 S protein led us to conclude that it does not exert its effects on the MMTV promoter through changes in chromatin remodeling or interaction with proteins binding to the E1A N terminus, CR1, or CR2. Mutations in multiple domains of CR3 completely abrogate E1A stimulation of both MMTV templates, indicating a mechanism in which the basal transcription machinery is targeted. Imaging analysis showed that E1A colocalizes with MMTV templates in vivo, indicating that it mediates its effect directly at the promoter rather than through an indirect mechanism.

Cells, Transfection, and Sorting—Cell line 1470.2 was derived from C127i mouse mammary adenocarcinoma cells. It contains multiple copies of the full-length MMTV long terminal repeat (LTR) driving expression of the chloramphenicol acetyltransferase gene in the context of an endogenous transcription factor.
The two E1A isoforms, 12 and 13 S, modulate transcription of various genes, but evidence indicates that they do so by disparate mechanisms (reviewed in Ref. 1). We assayed the effects of the isoforms expressed individually on the two MMTV templates. Typical results for the stably replicating MMTV template are shown in Fig. 3A. Summarized results from multiple independent experiments are shown in Fig. 3, B and C. Expression of the 12 S E1A protein has no significant effect on MMTV-chloramphenicol acetyltransferase RNA levels in either the presence or absence of Dex. The 13 S form induces MMTV-chloramphenicol acetyltransferase RNA plus or minus Dex to levels 3–4 times higher than observed in the absence of E1A expression. Thus, the stimulation of the MMTV template in organized chromatin by E1A is driven specifically by the 13 S isoform.

The two E1A isoforms had differential effects on the transient MMTV template, as shown in Fig. 4. Expression of the 12 S form led to a 65–70% repression of both basal and Dex-induced promoter activity. However, the 13 S form stimulated basal and Dex-induced promoter activity 5- and 25-fold, respectively. The intermediate stimulations observed in Fig. 2 when both isoforms were expressed are probably a combination of 13 S-induced activation and 12 S-induced repression. Thus, unlike the stably replicating MMTV template, both E1A isoforms modulate activity of the transient MMTV template. However, the 13 S E1A protein is able to stimulate transcription from the MMTV promoter in both structural contexts.

E1A Does Not Alter Chromatin Remodeling at the MMTV Template in Organized Chromatin—Genetic studies in yeast had shown that E1A expression blocked the ability of the Swi/Snf chromatin remodeling complex to participate in transcriptional activation of target genes (6). However, a link between SWI/SNF activity and E1A action has not yet been demonstrated in mammalian cells due to a lack of promoters known to require SWI/SNF function (29). Evidence indicates that the SWI/SNF complex is important for GR-induced transactivation in mammalian cells (30, 31). Association of the human SWI/SNF complexes with GR is correlated with the ability of the receptor to activate integrated MMTV promoter templates in human mammary adenocarcinoma cells (32). In addition, GR recruits SWI/SNF complexes containing the human Swi2 homolog, BRG1, to in vitro chromatin-assembled MMTV promoter sequences (33). The recruitment correlates with ATP-dependent remodeling of nucleosomes in the MMTV proximal pro-
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**FIG. 3.** E1A does not affect nuclease access at the stable MMTV template. Cells were transfected, treated, and sorted as described in the legend to Fig. 1, except that Dex treatment was 1 h in duration. Nuclei were isolated and digested with SacI. DNA was purified and digested to completion with DpnII prior to linear amplification of digestion products as described under “Experimental Procedures.” A diagram of the experimental design is shown in A. B, shows gel analysis of digestion products. Fractional cleavages by SacI are shown below each lane. Fractional SacI cleavage is calculated by dividing the intensity of the digestion fragment generated by SacI cleavage by the added intensities of the digestion fragments generated by DpnII and SacI (total cleavage) for each sample. C, shows percentage cleavage of SacI in nuclei from untreated cells that had been transfected with increasing amounts of E1A expression vector. The calculated fractional cleavage by SacI is converted to a percentage to yield percentage of SacI cleavage. ND, nontransfected cells. D, the change in percentage of SacI cleavage induced by Dex treatment in nontransfected cells as well as cells transfected with expression vectors for IL2R and increasing amounts of E1A. The change in percentage of SacI cleavage is calculated by subtracting the percentage of cleavage of untreated cells from that measured in Dex-treated cells for each transfection condition. The results shown are representative of two or three independent experiments.

**FIG. 4.** The 13 and 12 S isoforms target the transient MMTV template differentially. Cells were transfected and processed as described in the legend to Fig. 2, except that plasmids expressing either the 12 or 13 S isoforms were used in the place of pE1A-WT. A summary of results from at least five independent experiments is shown for untreated and Dex-treated cells in B and C, respectively.

moter region. Taken together, these results strongly suggest that Swi/Snf complexes catalyze the GR-induced structural transition at the MMTV promoter in organized chromatin. This remodeling event derepresses the promoter and allows previously excluded transcription factors to bind the promoter and participate in transcriptional activation (19).

GR-dependent chromatin remodeling at the MMTV template can be detected by restriction enzyme access assay and exonuclease block footprinting. The former measures nucleosome hypersensitivity via an SacI cleavage site in the GR-induced hypersensitive region (34). The latter measures the GR-induced binding of the ubiquitous transcription factor NF1 to the proximal promoter region (20). The results of the SacI access assay are shown in Fig. 5. Cells were transfected and sorted as described in Fig. 1. Nuclei were isolated and digested with SacI. After purification of the DNA, it was digested to completion with DpnII. Digestion products were detected by linear amplification from a radiolabeled, MMTV-specific primer as shown in Fig. 5A. A representative experiment is shown in Fig. 5B. An E1A-induced increase in fractional SacI cleavage in the absence of Dex might indicate a loosening of chromatin structure induced by E1A expression, independent of GR action. However, no such change was evident as seen in Fig. 5C. Activation of the GR by Dex treatment induces an increase in SacI cleavage as seen in Fig. 5B. The magnitude of that change in cleavage was also unaffected by increasing levels of E1A expression (Fig. 5D).

Dex-induced NF1 binding to the MMTV promoter was assayed in the presence and absence of E1A by an exonuclease block assay as shown in Fig. 6. It is clear that E1A expression does not cause NF1 to bind the promoter in the absence of Dex. Nor does E1A cause more NF1 to bind in the presence of Dex. These results allow us to conclude that E1A does not stimulate MMTV transcription by a mechanism involving chromatin remodeling and SWI/SNF activity.

**Domains Outside CR3 Have Varying Effects on E1A-induced Stimulation of MMTV Transcription—**The CR1, CR2, and N-terminal sequences of the E1A protein interact with a variety of proteins involved in transcription. CBP/p300 bind amino acid residues in the N terminus and CR1 regions (25, 35), whereas PCAF interacts with distinct residues in CR1 (4). Rb interacts with E1A through both CR1 and CR2 (35). It is not clear whether these interactions are also required for CR3-dependent stimulation of transcription. Therefore, we tested various E1A mutants for their ability to stimulate the MMTV promoter in organized chromatin as shown in Fig. 7. E1A proteins carrying point mutations in sequences required for interaction with either Rb (ΔRb) or PCAF (ΔPCAF1 and ΔPCAF2) were
able to stimulate both basal and Dex-activated transcription at the MMTV promoter at least as well as wild type E1A (E1A WT), as shown in Fig. 7, A and B. However, the degree of stimulation was increased. All of these mutants stimulated basal transcription better than E1A WT (Fig. 7A). In the presence of Dex, ARb did not stimulate promoter activity to a greater degree than E1A WT, but both p300. RG2 carries a arginine to glycine mutation in the second amino acid of E1A and has been shown by immunoprecipitation to be deficient in the ability to bind p300 (35). The transcription adapter motif mutant carries several alanine substitutions in a CR3 domain shown to be important for binding of CBP (25). These mutants were all able to stimulate basal MMTV transcription to the same extent as E1A WT as shown in Fig. 7C. In the presence of Dex (Fig. 7D), both E1A WT and the transcription adapter motif mutant stimulated the promoter to the same extent. However, RG2 was less efficient than E1A WT but did not inhibit stimulation entirely. Although various domains outside CR3 affected the degree of E1A-induced stimulation, none of these domains was absolutely required for stimulation.

Multiple Domains of CR3 Are Necessary for Stimulation of the MMTV Promoter—The CR3 region of E1A has been shown to interact with several transcriptionally important proteins. The sequences in the C terminus of CR3 have been shown to interact with both transcription factors and several TAFs, TAF1125, TAF11135/110, and TAF55 (13–15). This subdomain is thought to play a role in the targeting of E1A to various promoters through interactions with transcription factors such as ATF2, Sp1, and upstream stimulatory factor (36). A centrally located Cys4 zinc finger interacts with TBP and is crucial for E1A-induced stimulation of adenovirus promoters (12). The N-terminal portion of CR3 facilitates interaction with hTAF135 (15). We assayed several E1A mutants carrying small deletions in each of the three subdomains of CR3. E1A-induced stimulation of basal and activated transcription was completely abolished by each of the three mutations at either the stable MMTV template (Fig. 8, A and B) or the transient template (Fig. 8, C and D). The results show that all three domains are absolutely required for transcriptional stimulation of the MMTV promoter and suggest that the target of E1A action is the basal transcription machinery.

E1A Colocalizes with the MMTV Locus in Vivo—If E1A activates the MMTV promoter through the basal machinery, it is likely to be located at the promoter rather than exerting its effect by an indirect mechanism. To determine whether E1A might be acting directly at the MMTV promoter in organized chromatin, we used a cell line, 3617, containing 200 tandemly integrated copies of an MMTV-ras transcription unit and expressing GFP-tagged GR. This array of transcription units can be visualized by fluorescence microscopy in the presence of Dex through an accumulation of GFP-GR (26). We transfected these cells with the E1A expression vector. After fixation, E1A protein was visualized through indirect immunofluorescence using a primary antibody against E1A and Texas Red-conjugated secondary antibodies. MMTV sequences were visualized through fluorescence from the GFP-GR. Colocalization of E1A and GFP-GR signals at the MMTV array was easily detectable in a significant fraction of cells. Two representative examples are shown in Fig. 9. The MMTV array is often located near nucleoli and is present at 1–2 copies/cell (26). The lower panels show a cell that contains two copies as seen by two intense spots containing GFP-GR. E1A colocalizes with both. These results further support our hypothesis that E1A stimulates the MMTV promoter through direct interaction with basal transcription machinery at the promoter. We were not able to determine by imaging whether E1A colocalizes with MMTV sequences in the absence of Dex due to several factors. First, we do not have a marker for the MMTV array in the absence of Dex. Second, the array appears to have a compacted chromatin structure in the absence of Dex and decondenses after Dex treatment (37). This compaction may restrict access of antibodies to proteins bound to the promoter. Thus, detection of proteins at the array in the condensed state by immunofluorescence has proven to be difficult.

DISCUSSION

In our study, we have established that the MMTV promoter is a target for the action of E1A proteins in both basal and activated states. The 12 S E1A isoform targets the MMTV promoter in a manner influenced by its nucleoprotein structure, whereas the 13 S form targets the promoter regardless of its chromatin configuration, significantly stimulating promoter activity. When both proteins are expressed, the stimulatory effect is predominant. We have also investigated the mechanism by which E1A activates the MMTV promoter. The evidence indicates that E1A mediates this effect through interactions with the basal transcription machinery directly at the promoter.

The 12 S E1A protein potently represses promoter activity only at the transient MMTV template. It is without effect at the MMTV template in organized chromatin. We have previously reported a number of functional differences between the two templates (38). They respond differentially to both cAMP (39).
and progesterone receptor signaling (27, 40). In addition, the GR activates the two templates through disparate mechanisms (19). These functional differences strongly indicate that distinct sets of transcriptional cofactors may work in the two nucleoprotein environments of the MMTV promoter. Some of those factors uniquely required for promoter activity at the transient MMTV template may be sequestered or inactivated by the 12 S protein. Inhibition of enhancer activity by the 12 S protein is thought to be due to sequestration of CBP/p300 or Rb (reviewed in Refs. 1 and 2). In addition, E1A has been shown to inactivate histone acetyltransferase activity of CBP, p300, and PCAF (41–43).

The 13 S E1A protein significantly stimulates the MMTV promoter regardless of its nucleoprotein structure. It also targets the promoter whether it is activated or not. The magnitude of stimulation at the stable MMTV template is similar plus or minus Dex, about 3-fold. At the transient template minus Dex, the 13 S protein stimulates the promoter to about the same extent as observed at the stable template. However, in the presence of Dex, the degree of stimulation at the transient template is much greater, about 25-fold. This observation implies that the 13 S E1A protein may be having additional effects on cofactors recruited by the activated GR. This Dex-dependent effect is not observed at the stable template and is consistent with our findings that the GR does not activate the two templates by the same mechanism (E. K. Kecta and C. L. Smith, unpublished data). E1A has been shown to have effects on ligand-dependent activation by other nuclear receptors. The 13 S E1A protein stimulates activation induced by the thyroid and retinoic acid receptors (44, 45). In contrast, E1A has been shown to inhibit progesterone receptor-dependent transactivation (46).

Chromatin remodeling is an important component in gene regulation. ATP-dependent remodeling complexes such as SWI/SNF, NURF, RSC, and NURD have been shown to alter the relationship between DNA and histone octamers in nucleosomes by a mechanism that is not completely understood (47). GR-induced activation of the MMTV promoter in organized, repressed chromatin involves a chromatin remodeling event characterized by the formation of a nuclease hypersensitive region (21, 22). Evidence indicates that SWI/SNF may play a role in mediating this event at the MMTV promoter (32, 33). E1A has been reported to have a functional interaction with the SWI/SNF complex in yeast (6). E1A expression in yeast specifically inhibited the transcriptional activation of genes dependent on SWI/SNF activity in a manner dependent on expression of various SWI/SNF components. Sequences in the amino-terminal region of E1A including both the N terminus and CR1 were required for mediation of this effect (7). Because of this link between E1A action and SWI/SNF function, we were interested in knowing whether E1A stimulates the stable MMTV template through chromatin remodeling. Our experiments showed, however, that E1A does not change nuclease access to the MMTV promoter region in the presence or absence of glucocorticoids. Thus, the E1A-induced stimulation of the MMTV promoter in organized chromatin does not involve changes in chromatin structure. This is consistent with the fact that the 13 S protein stimulates the MMTV promoter independent of its

![Image](https://www.jbc.org/)

**FIG. 8.** Mutations in the CR3 region completely abrogate E1A stimulation of both MMTV templates. Cells were transfected with or without expression vectors for E1A and various CR3 deletion mutants. For the stable MMTV template, cells were treated for 3 h with 100 nM Dex and sorted by MACS prior to isolation and analysis of RNA. For the transient MMTV template, transfections included pLTRluc. Transfected cell cultures were treated for 6 h with 100 nM Dex prior to harvest and preparation of extracts for luciferase analysis. Result summaries from at least three independent experiments carried out with untreated (A and C) or Dex-treated (B and D) cells and analyzed for promoter activity at the stable (A and B) and transient (C and D) MMTV templates.

**FIG. 9.** E1A colocalizes with MMTV sequences in vivo. Cell line 3617 was transfected with plasmids expressing E1A WT (upper three panels) or just the 13 S isoform (lower three panels). After treatment with Dex for 3 h, cells were fixed and exposed to an antibody against E1A. After exposure to Texas Red-conjugated secondary antibodies, the cells were analyzed by fluorescence microscopy to visualize GFP-GR (green, rightmost panels) and E1A (red, center panels). The overlay of red and green signals is shown in the leftmost panels.
nucleoprotein configuration and points toward a common mechanism of activation.

E1A proteins carrying mutations in the N terminus, CR1, or CR2 failed to abrogate stimulation. In fact mutations in the binding sites for Rb or PCAF actually enhanced stimulation. This resembles the PEPCk gene, which is also activated by 13 S E1A. Klemm et al. (48) reported that mutation of the Rb binding site in E1A enhanced this activation. In addition, overexpression of Rb alone resulted in stimulation of the PEPCk promoter. The authors speculated that E1A and Rb activated the PEPCk promoter by different mechanisms but that E1A blocked Rb action at the promoter. It is not known whether Rb or PCAF are required for MMTV transcription; both are known to potentiate GR action (49, 50). However, we observe the increased stimulation of MMTV promoter activity by the Rb and PCAF mutants in both the presence and absence of Dex. It is likely that E1A forms a number of distinct complexes in vivo. Perhaps the loss of binding to Rb or PCAF effectively increases the concentration of E1A available to participate in the stimulation of transcription. The E1A mutants that do not bind CBP/p300 do not increase the degree to which the MMTV promoter is activated relative to E1A WT. In the case of the RG2 mutant, stimulation in the presence of glucocorticoids is decreased relative to E1A WT but not abolished. Sequences at the N terminus may be partially required for activation when the GR is present at the promoter. Alternatively, other factors may associate with this region in the absence of CBP/p300 binding (51), and their presence may be inhibitory to GR action at the promoter.

Our data indicate that the CR3 region alone is essential for stimulation of the MMTV promoter. In this respect it resembles E1A stimulation of both the PEPCk gene (48) and cytomegalovirus early promoter (52). The CR3 region consists of a centrally located Cys_a zinc finger with 8–10 amino acids on either side. We tested E1A mutants containing small deletions in each of these areas. The Δ140–146 mutant contains a deletion in amino acids N-terminal to the zinc finger and has been shown to be compromised for binding to TAF(_135) (15). Amino acids in the zinc finger critical for binding TBP are deleted in the Δ169–174 mutant (12). The third deletion mutant, Δ180–188, is missing amino acids C-terminal to the zinc finger that are important for interactions with TAF(_110/135) (14, 15), TAF(_250) (14), and transcription factors such as ATF2 (36). In addition, various amino acids in both the zinc finger and the C-terminal domain are necessary for E1A binding to the mediator complex (16). All three of these deletions completely abolished stimulation of the MMTV promoter by E1A. The involvement of the N-terminal amino acids in activation was surprising, since point mutations throughout this area had no effect on activation of the adenovirus E3 promoter (12). Thus, the structural integrity of the entire CR3 region is necessary for E1A function and at the MMTV promoter. The results imply that E1A makes multiple contacts with components of the basal machinery to activate the promoter.

Our imaging analysis shows that E1A colocalizes with MMTV sequences in vivo, lending further support to our hypothesis that E1A works directly at the MMTV promoter rather than through an indirect mechanism. This raises the question of how E1A is targeted to the MMTV promoter. It does not contain binding sites for CREB/ATF2, Sp1, or upstream stimulatory factor. In preliminary experiments with mutant MMTV promoter constructs, we were not able to identify a single transcription factor binding site critical for E1A action (data not shown). Like the adenovirus E2 promoter, the MMTV promoter may contain more than one target for E1A, each of which could function independently (1). It is also possible that E1A is attracted to the promoter by the basal transcription machinery. Many E1A target promoters do not share common sequence elements. It is not clear whether the basal transcription machinery at each RNA polymerase II-transcribed promoter has exactly the same components or the same conformation. E1A may target a specific subset of this machinery.

Our study is unique in that we have addressed the issue of whether the chromatin structure of a promoter plays a role in its response to E1A. We have ascertained that, in the case of the MMTV promoter, nucleoprotein configuration influences the response to the 13 S protein but not the 13 S protein. Analysis of the mechanism by which the 13 S protein stimulates MMTV promoter activity shows that it does not involve chromatin remodeling, although the SWI/SNF complex has been implicated in both activation of the MMTV promoter and E1A effects in yeast. The link between E1A and SWI/SNF function may not extend to mammalian systems, although more SWI/SNF target promoters remain to be identified and tested. Regions outside CR3 are not strictly required for activation of the MMTV promoter although they interact with transcriptional coactivators. However, all the subdomains in the CR3 region are essential for stimulation of the MMTV promoter, indicating a activation mechanism involving multiple contacts with components of the basal transcription machinery.

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Regulation of MMTV Transcription by E1A

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