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Biochemistry

DNA octamer element can confer E1A trans-activation, and adenovirus infection results in a stimulation of the DNA-binding activity of OTF-1/NFIII factor

(transcription control/DNA replication)

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ABSTRACT **Adenovirus E1A-dependent trans-activation of viral transcription involves the utilization and alteration of multiple sequence-specific transcription factors. Cellular genes are also activated by E1A, one example being the immunoglobulin heavy-chain locus when assayed by transfection into fibroblast cells. We have explored the basis for the E1A-dependent activation of this cellular transcription unit. We demonstrate that the ATTTGCAT ("octamer") element found in the heavy-chain enhancer and promoter is a target for E1A trans-activation since this sequence can confer inducibility to the normally unresponsive simian virus 40 early promoter. In addition, adenovirus infection stimulates the DNA-binding activity of the ubiquitous octamer-specific factor, OTF-1, and we presume that this is the basis for the stimulation of transcription. Although there are no octamer elements in the adenovirus genome that are known to be important for transcription, there are octamer elements in the viral terminal repeat sequences. These elements bind the NFIII factor and are important for the initiation of DNA replication. Since the NFIII factor has been shown to be identical to OTF-1, we suggest that the stimulation of OTF-1/NFIII activity during an adenovirus infection may be important for efficient initiation of adenovirus DNA synthesis.**

The use of animal viruses, and especially the DNA tumor viruses, has been invaluable in developing an understanding of eukaryotic gene regulation. Perhaps because of the simplicity of their genomes but also due to the genetics that viruses bring to a mammalian system, these viral systems have provided a unique path to exploring the basis for control of transcription factor activity. In each case, genetic analysis has defined a gene or genes that act in trans to stimulate transcription of a group of additional viral genes (1). One such viral trans-activating gene that has been studied extensively is the E1A gene of adenovirus. The expression of other early genes of adenovirus is dependent on the expression of the E1A gene (2–4), and various studies have shown that this trans-activation is not limited to the early adenovirus genes but extends to a variety of genetically unrelated viral and cellular genes (5, 6). The finding that E1A does not bind to DNA in a sequence-specific manner (7) and that there is no single target sequence shared by all E1A-inducible promoters suggested that E1A does not induce transcription by direct binding to the promoters.

Indeed, it is now firmly established that trans-activation by E1A is mediated through cellular transcription factors (1, 8). Moreover, it appears that multiple promoter-specific factors must be targeted as intermediates in the trans-activation event. The E2F factor was initially identified on the basis of an increased DNA-binding activity upon adenovirus infection and the fact that binding required functionally important promoter sequences (9, 10). The same strategy identified an E4-binding protein termed E4F (11). Other studies have identified the TFIIIC transcription factor (12, 13) and a TATA-specific factor (14, 15) as likely to be involved in E1A control. Finally, recent experiments indicate that the AP1 factor is induced by adenovirus infection (16) and, in view of the previous mutagenesis experiments (17), may be important for trans-activation of the E3 promoter.

There are other cases, however, where E1A-dependent trans-activation cannot readily be accounted for in terms of the factors already described. One particularly intriguing case is the control of the immunoglobulin heavy-chain locus since, depending on the cell type, E1A can either activate (18) or repress (19) transcription. We have pursued the basis for this control by analyzing factors in extracts of virus-infected cells that interact with regulatory elements of the immunoglobulin enhancer. Although we find no evidence for an alteration that might explain the negative control by E1A, we do find that the ubiquitous OTF-1 factor, which binds specifically to the 8-base-pair sequence 5′-ATTTGCAT-3′ ("octamer"), is increased in adenovirus-infected fibroblasts. This correlates with the finding that the octamer element can confer E1A control on a heterologous promoter.

MATERIALS AND METHODS

Cells and Virus. CV-1 monkey kidney cells were grown as monolayers in Dulbecco's modified Eagle's medium (DMEM) containing 5% bovine calf serum. F9 mouse embryonal carcinoma cells were maintained in the same medium containing 10% fetal bovine serum and were induced to differentiate by the addition of retinoic acid (0.1 μM) and dibutryl cAMP (1 mM) as described (20). The growth and purification of wild-type adenovirus 5 (Ad5) and the E1A-deficient mutant dl312 have been described (21).

Infection and Whole Cell Extract Preparation. Whole cell extracts were prepared from CV-1 cells infected for 18 hr with 4000 particles of wild-type Ad5 per cell, as described (11). Extracts were made from undifferentiated F9 cells, F9 cells differentiated for 5 days, or F9 cells differentiated for 4 days and then infected with Ad5 for 18 hr by the same protocol.

Gel Shift Assay. The *Dde* I–*Hin*fI fragment of the immunoglobulin heavy-chain enhancer was 3′-end-labeled by DNA polymerase Klenow fragment and used as a probe. A similar synthetic oligoncleotide fragment with the octamer sequence mutated as shown in Fig. 1 was used for competitions. Gel shift assays were performed essentially as described (22), using 10 μg of whole cell extract and 3 μg of poly(dI–dC)·poly(dI-dC) as competitor.

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Abbreviations: Ad5, adenovirus 5; CAT, chloramphenicol acetyltransferase; SV40, simian virus 40.

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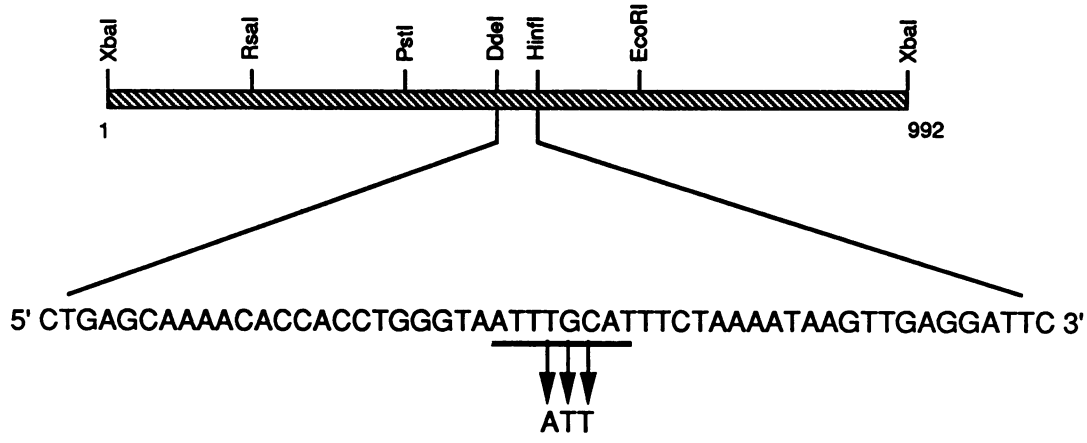

FIG. 1. Schematic of the immunoglobulin heavy-chain enhancer (in a 992-base-pair *Xba* I restriction fragment) and the sequence of the octamer-containing DNA fragment used for binding assays. The octamer sequence is underlined. The nucleotide changes in the octamer mutant are indicated.

Plasmids. The chloramphenicol acetyltransferase (CAT) vector pBSCAT2, (R. Rooney and J.R.N., unpublished data) contains the CAT gene under the control of the simian virus 40 (SV40) early promoter element. The octamer or the mutant octamer fragments were cloned into the *Bgl* II site of pBSCAT2. The E1A-expressing plasmid pE1A has been described (23).

Transfection Assays. Cotransfections were done by the DEAE-dextran method (24). The corresponding target plasmid (4 μg) and 3 μg of either pE1A as trans-activator or pGEM (Promega) as control were transfected on 10-cm dishes. The cells were harvested after 44–48 hr. CAT assays were performed as described (25).

RESULTS

Adenovirus Infection Stimulates OTF-1 Binding Activity. Previous experiments showed that the immunoglobulin enhancer element could confer E1A stimulation if assayed in fibroblast cultures (18), whereas adenovirus infection of lymphocytes resulted in a repression of transcription of the heavy-chain locus (19). To probe the basis for this dichotomy of E1A control, we have examined extracts from adenovirus-infected cells, either of lymphoid origin or fibroblasts, for alterations in factors that interact with the heavy-chain enhancer. This approach has been fruitful in previous work aimed at understanding the basis for E1A trans-activation of viral transcription, leading to the identification of the E2F and E4F transcription factors that are induced by viral infection and that interact with promoter regulatory sequences (9, 11). We therefore reasoned that the repression event might be a reflection of an increase in a negative factor or the loss of a positively acting factor.

Although we could not detect changes in adenovirus-infected lymphocytes that might correlate with E1A-mediated repression, there was a clear difference in a factor binding to the enhancer detected in CV-1 fibroblast extracts after infection with wild-type adenovirus. Whole cell extracts were prepared from mock-infected CV-1 cells or cells infected with Ad5 for 18 hr and were assayed for binding to various fragments derived from the enhancer. The adenovirus-infected cells showed a substantial increase in the level of what appeared to be the OTF-1 binding activity, as judged by the gel mobility pattern of an octamer-containing fragment (Fig. 2). The specificity of binding, and thus the indication that this was OTF-1, was demonstrated by competition with a synthetic oligoncleotide representing the wild-type octamer sequence (Octa). In contrast, an oligoncleotide with mutations in the octamer sequence (Octa*) or a fragment from the adenovirus E2 promoter that does not contain an octamer element failed to compete. Based on the binding specificity as well as the gel mobility of the complex, we thus conclude that adenovirus infection results in a stimulation of OTF-1 DNA-binding activity.

Cellular E1A-Like Control of OTF-1. The embryonal teratocarcinoma cell line F9 appears to possess an activity similar in nature to E1A with respect to transcription control. That is, F9 cells will complement an E1A-deficient virus for activation of early viral transcription, whereas this activity is lost upon differentiation (26). This activity is also reflected in

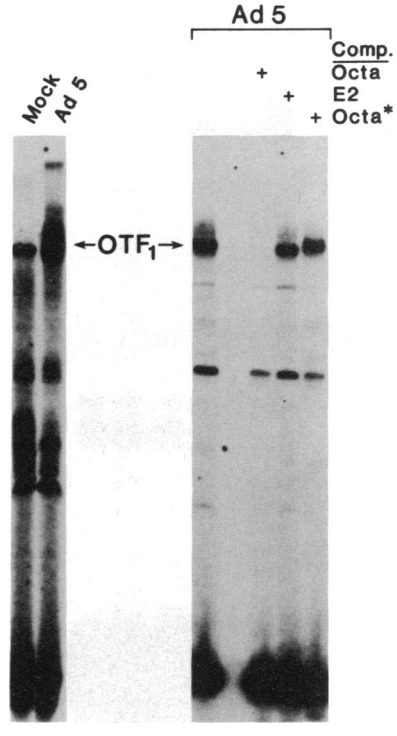

FIG. 2. Adenovirus infection stimulates OTF-1 binding activity as detected in gel retardation assays. (*Left*) CV-1 cells were mock-infected or infected with wild-type Ad5 for 18 hr. Whole cell extracts were prepared and analyzed for OTF-1 binding activity by gel retardation. (*Right*) The specificity of OTF-1 activity in the extract of Ad5-infected cells was determined by competition with the homologous-sequence probe (Octa), an oligoncleotide containing the E2F binding sites in the adenovirus E2 promoter (E2), or an oligoncleotide containing a mutated octamer sequence (Octa*).

the control of levels of the E2F and E4F transcription factors that are believed to play a role in E1A trans-activation (27, 28). Consistent with these results and the finding that adenovirus infection of CV-1 cells induced OTF-1, we found that OTF-1 levels were also regulated during F9 cell differentiation. The level of OTF-1 in the F9 stem cells was high but then declined markedly upon differentiation (Fig. 3). In addition, another octamer-specific factor was detected in F9 cells, designated OTF-3 in Fig. 3, and this is most likely the same as the Oct-3 factor or NF-A3 factor recently described (29, 30). This activity also declined upon retinoic acid/cAMP-induced differentiation.

Infection of the differentiated F9 cells with Ad5 resulted in a stimulation of the OTF-1 activity, similar to the result in CV-1 cells, bringing the level back to nearly that found in the undifferentiated F9 cells. Interestingly, this was a specific event, since the F9-specific OTF-3 activity was not altered by adenovirus infection (Fig. 3). These results thus demonstrate that the OTF-1 factor is regulated under several conditions consistent with control by E1A.

E1A Can Trans-Activate via the Octamer Element. Borrelli *et al.* (18) have shown that E1A can trans-activate transcription dependent upon the immunoglobulin heavy-chain enhancer. Given our finding that the OTF-1 octamer-specific factor was activated by infection, the obvious explanation for the findings of Borrelli *et al.* is that the octamer element was a target for the trans-activation event. As a direct test, we have assayed for the importance of the octamer sequence to confer E1A control on a normally unresponsive promoter. A previous report (22) showed that the SV40 early promoter was not stimulated by adenovirus E1A and that the E2F elements from the E2 promoter could confer E1A responsiveness on this construct (22). Using this same test system, we have determined the role of the octamer element in conferring E1A control. Either an octamer-containing fragment or a fragment bearing a mutation in the octamer element was cloned upstream of the SV40 promoter controlling a CAT gene. These vectors were cotransfected with the same amounts of either pGEM as control or pE1A as trans-activator (Fig. 4). The SV40 promoter containing the intact octamer sequence was stimulated 10-fold by E1A. In contrast, stimulation of the promoter containing the mutant octamer was minimal, less than 2-fold above basal level. This experiment therefore demonstrates that the octamer element is a target for E1A control, which we presume to be the result of the increase in OTF-1 DNA-binding activity.

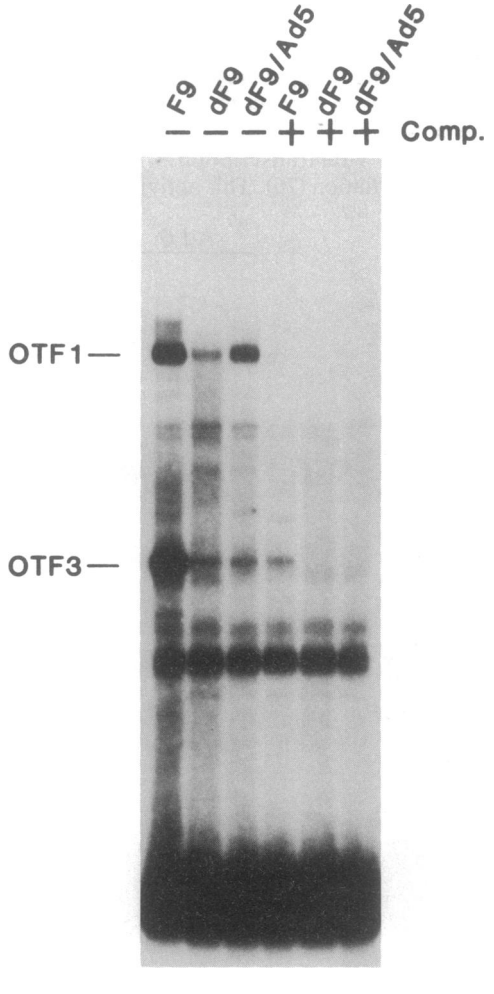

FIG. 3. Control of OTF-1 binding activity during differentiation of F9 teratocarcinoma cells. Whole cell extracts were prepared from F9 cells, from F9 cells induced to differentiate with retinoic acid and dibutyryl cAMP (dF9), and from differentiated F9 cells that were infected with Ad5 (dF9/Ad5). Binding was assayed with the octamer-containing DNA probe. OTF-1 binding in each extract was measured in the absence (−) or presence (+) of nonradioactive octamer competitor.

DISCUSSION

Our results demonstrate that the octamer element of the immunoglobulin heavy-chain enhancer is a target for E1A-mediated trans-activation and that this correlates with an increase in the level of the transcription factor OTF-1. Although the precise biochemical mechanism by which E1A induces OTF-1 activity is not clear, we believe it is likely to be an event similar to that for the other factors regulated in an E1A-dependent manner. Analysis of the activation of the E2F (31) and E4F (28) transcription factors has revealed a role for phosphorylation in the control of DNA-binding activity. The DNA-binding activity of each factor is sensitive to phosphatase digestion and each can be reactivated by incubation with an extract from virus-infected cells much more efficiently than with an extract from uninfected cells. Given the common control of these three factors (E2F, E4F, OTF-1) in differentiating F9 cells as well as in an adenovirus infection, it seems reasonable to suggest that each may be regulated by similar mechanisms.

Of course, this is not the first instance of targeting of the OTF-1 factor by a viral regulatory protein. The induction of the immediate early genes of herpes simplex virus by the virion-encapsidated VP16 trans-activating protein is mediated through the OTF-1 factor (32). The activation involves a direct association of VP16 with OTF-1 (32–35) and appears to have two consequences. There is a change in DNA-recognition properties to include the TAATGARAT sequence, and the VP16 protein appears to contribute a strong transcriptional activity domain. We have found no evidence for a similar direct association of E1A with OTF-1; E1A antibodies did not alter the OTF-1–DNA complex in a gel shift assay, and we could not deplete OTF-1 activity from extracts with an E1A antibody (data not shown). Moreover, the gel mobility of the complex detected in virus-infected cells was identical to that in uninfected cells. Thus, we have no reason to believe that the mechanism utilized in an adenovirus infection is similar to that brought about by VP16. Rather, it appears that adenovirus infection results in an increase in the level of active OTF-1.

In the context of adenovirus transcription control, it is difficult to envision a rationale for activation of the ubiquitous OTF-1 octamer factor. There is no evidence that any of the E1A-induced promoters contain an octamer element and thus utilize the OTF-1 factor. It is possible that the induction of OTF-1 is directed at cellular genes so as to alter the cell environment for a more productive infection, but this is

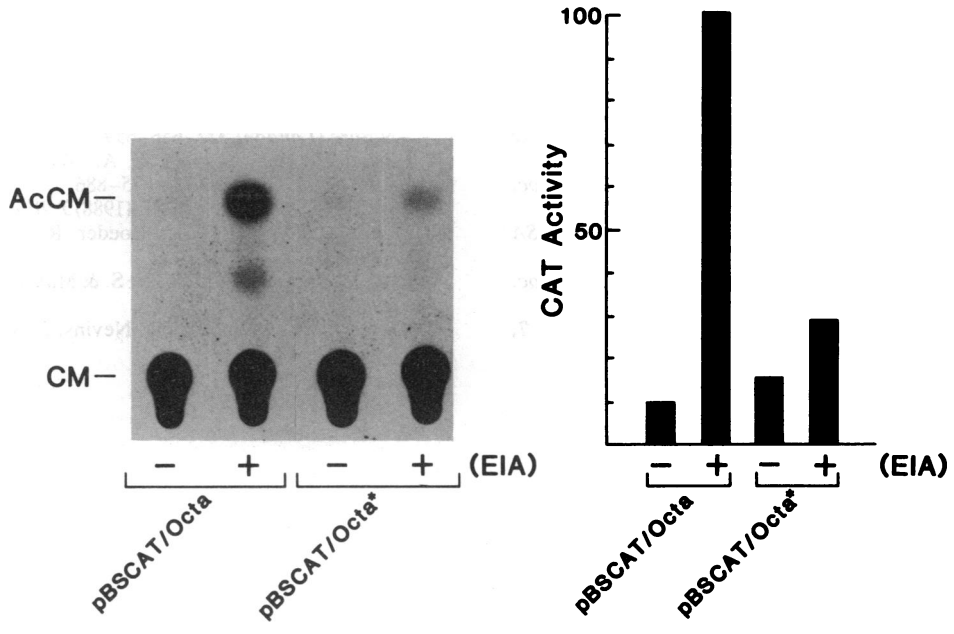

FIG. 4. The octamer element can confer E1A-dependent trans-activation. CV-1 cells were transfected with CAT plasmids that were under the control of the SV40 early promoter and that contained the wild-type octamer element (pBSCAT/Octa) or the mutant octamer element (pBSCAT/Octa*) depicted in Fig. 1. These two plasmids were transfected with (+) or without (−) the E1A-expressing plasmid pE1A. Extracts were prepared after 48 hr and assayed for CAT activity. (*Left*) Thin-layer chromatographic separation of chloramphenicol (CM) and its acetylated product (AcCM). (*Right*) Quantitation of the CAT assays. Activities were normalized to the pBSCAT/Octa, + pE1A value (100%).

difficult to assess. However, we believe the functional significance of this event may relate to a role for the octamer factor in adenovirus DNA replication rather than in transcription control. Recent experiments (36) have shown that the OTF-1 factor is identical to a factor termed NFIII, previously defined as an essential component for the initiation of adenovirus DNA replication. NFIII binds to a sequence in domain C of the adenovirus origin of replication and stimulates initiation of adenovirus DNA synthesis *in vitro* (37–39). Considering that OTF-1 is an important component for initiation of adenovirus DNA synthesis as well as the fact that as replication proceeds the DNA copy number greatly expands, we suggest that the increase in OTF-1 levels is important to allow efficient DNA synthesis.

We imagine that this would be particularly important in a quiescent cell where the level of factors involved in DNA synthesis may be low. Indeed, it is interesting that OTF-1 is also required for cell cycle-regulated histone H2B transcription (40) and one study has indicated that OTF-1 levels increase upon stimulation of cell proliferation (41). Thus, in a quiescent cell that adenovirus would normally infect *in vivo*, the level of available OTF-1 might be low and thus limiting for viral replication. Then, as a result of E1A expression, OTF-1 levels would rise, thereby permitting efficient replication. This scenario is also consistent with recent findings concerning control of the E2F transcription factor. This factor, which is required for transcription of the early E2 promoter, is also regulated by cell proliferation (42). Possibly, the virus has evolved to make use of factors that are involved in proliferation, perhaps because these factors are ubiquitous, being required for transcription and DNA synthesis in virtually every cell in the body. The evolution of an activity such as E1A might then be rationalized as a need to increase the level of such factors to improve viral transcription and replication.

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