MOLECULAR AND CELLULAR BIOLOGY, Nov. 1990, p. 5857–5864
0270-7306/90/115857-08$02.00/0

Vol. 10, No. 11

Differential Effects of the Adenovirus E1A Oncogene on Members of the AP-1 Transcription Factor Family

HANS VAN DAM,[1] RIENK OFFRINGA,[1] INGEBORG MEIJER,[1] BERND STEIN,[2] ALIDA M. SMITS,[1] PETER HERRLICH,[2] JOHANNES L. BOS,[1] AND ALEX J. VAN DER EB[1]*

Laboratory for Molecular Carcinogenesis, Sylvius Laboratories, University of Leiden, P.O. Box 9503, 2300 RA Leiden, The Netherlands,[1] and Kernforschungszentrum Karlsruhe, Institut für Genetik und Toxikologie, D-7500 Karlsruhe 1, Federal Republic of Germany[2]

Received 15 June 1990/Accepted 13 August 1990

The adenovirus early region 1A (E1A) oncogene interferes with the expression level and activity of the AP-1 transcription factor family. E1A abolished the transactivating function of AP-1 (Jun/Fos), which binds to the 12-*O*-tetradecanoylphorbol-13-acetate-responsive element of the collagenase gene (collTRE). In contrast, the activity of another member of the AP-1 family that binds to the c-*jun*TRE was not repressed. The mRNA expression of the c-*jun* gene was, in fact, strongly elevated in various cell types expressing the E1A gene of either adenovirus type 5 (Ad5) or Ad12. The regulation of the *junB* gene by adenovirus E1A, on the other hand, depended both on the cell type and on the transforming adenovirus serotype. The fact that E1A-induced alterations in the repertoire of AP-1 transcription factors depend on its transforming domain in conserved region 1 suggests that the effects are relevant for the transformation process.

Early region 1A (E1A) plays a pivotal role in the transformation of cells by human adenoviruses. The E1A gene encodes nuclear phosphoproteins that exhibit various activities, including positive and negative regulation of gene expression and the stimulation of cellular DNA synthesis (5, 7, 11, 30). Extensive mutational analysis has demonstrated the existence of three major functional domains within this gene, conserved regions (CR) 1, 2, and 3. Of these domains, CR1 and CR2 are essential both for the transforming capacity of E1A and for the repression by E1A of several viral and cellular enhancers (14, 17, 19, 26). Furthermore, these domains are involved in the association of E1A with cellular proteins, e.g., p105-RB, the product of the retinoblastoma susceptibility gene, and a 300-kDa protein of unknown function (9, 39, 40). This suggests that gene regulation and direct interaction of E1A with cellular proteins are important aspects of adenovirus transformation.

In previous studies we have shown that E1A, through CR1, represses several mitogen-inducible genes, including the growth-related genes c-*myc* and JE and the genes which code for the proteases stromelysin and collagenase type I (20a, 34). Studies on the repression of the latter gene demonstrated that E1A downregulates the activity of the collagenase promoter through its 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-responsive element (collTRE). The collTRE is a high-affinity binding site for the transcription factor AP-1, a heterodimeric complex consisting of the proteins Jun and Fos (3, 8, 20a, 25). E1A was found to inhibit the transcriptional activation function of AP-1 without decreasing the synthesis of its constituents Jun and Fos and without inhibiting its binding to the collTRE in vitro. Rather, AP-1 binding activity was elevated in extracts from several E1A-transformed cells. We now explore the cause of this paradoxical change in AP-1 function (increased binding versus reduced transactivation).

In this paper, we report on the stimulation by adenovirus E1A of the c-*jun* and *junB* genes, which encode constituents of the AP-1 transcription factor family (6, 20). Analysis of the E1A-dependent stimulation of the c-*jun* gene indicated that several sequences within the c-*jun* promoter are involved. The contribution to E1A-dependent stimulation of one of these sequences, termed c-*jun*TRE (2), depends on the promoter context in which this element is presented, since the activity of a minimal synthetic promoter carrying the c-*jun*TRE was neither stimulated nor repressed by E1A. Interestingly, under similar conditions the activity of the collTRE was severely inhibited by E1A. Our data, therefore, strongly suggest that TPA induces different transcription factors to bind to the collTRE and c-*jun*TRE, the latter of which is not sensitive to repression by the E1A oncogene.

MATERIALS AND METHODS

Cell lines and cell culture. Human embryonic retinoblast (HER), baby rat kidney (BRK), and normal rat kidney (NRK) cell lines transformed by adenovirus type 5 (Ad5), Ad12, simian virus 40 (SV40), polyomavirus, or *ras* oncogenes have been described previously (21, 32, 34, 35). These cells were routinely cultured in Eagle minimal essential medium (MEM) supplemented with 1 mg of glucose per ml, 1 μg of biotin per ml, 300 μg of glutamine per ml, antibiotics, and 10% fetal calf serum (FCS). HeLa tk$^-$ cells were grown in Dulbecco modified Eagle medium supplemented with 10% FCS.

RNA analysis. RNA was isolated from rapidly growing subconfluent cultures whose medium had been changed 1 day prior to isolation. RNA isolation and Northern (RNA) blotting analysis with stromelysin and human elongation factor 1 (HEF) probes were performed as described elsewhere (21, 34). For the detection of *jun* mRNA, mouse c-*jun* (R. Bernards, unpublished results) and *junB* probes (23) were used.

Transient transfection and plasmid constructs. Transfection of plasmid DNA into HeLa tk$^-$ cells and chloramphenicol acetyltransferase (CAT) assays were performed as described previously (1). CAT activity was quantified by liquid scintillation counting of excised sections of thin-layer plates and expressed as percent conversion of [^{14}C]chloramphenicol

* Corresponding author.

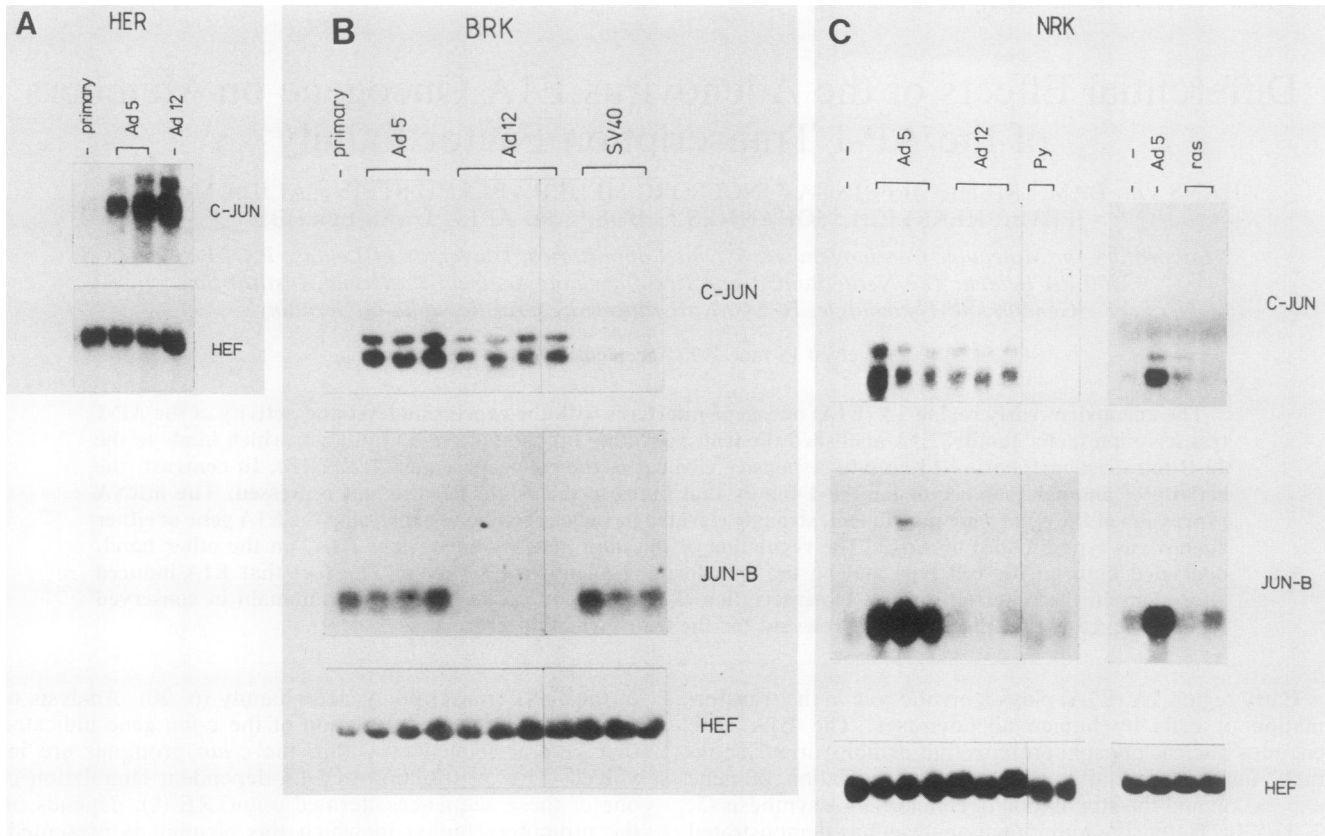

FIG. 1. Effects of adenovirus transformation on c-*jun* and *junB* mRNA expression. Total cytoplasmic RNA prepared from subconfluent, growing cell cultures was analyzed by Northern blotting. Each lane contains 20 μg of RNA. The filters were hybridized to c-*jun*- and *junB*-specific probes and to a HEF probe as a control. The following cell lines were analyzed. (A) Primary HER cells, Ad5 E1-transformed HER clones C2 and C5, and Ad12 E1-transformed HER clone C4. (B) Primary BRK cells; Ad5 E1-transformed BRK clones C1, C2, and C4; Ad12 E1-transformed BRK clones 2C1, 2C2, C16, and C18; and SV40 early-region-transformed BRK clones C1, C21, and C22. (C) Untransformed NRK cells (lanes −), Ad5 E1A-transformed clone 1-1, Ad5 E1 clones 1-2 and 4-1, Ad12 E1A clone 1-2, Ad12 E1 clones 2-4 and 4-3, polyomavirus-transformed clones 2-1 and 2-2 (lanes Py), Ad5 E1 clone 1-2, EJ*ras*-transformed clones R6 and R11. All cell lines have been analyzed for the correct expression of the oncogenes, as has been described previously (21, 32, 34, 35).

col to its acetylated derivatives. The mutant E1A genes have been described elsewhere (26). For the transient cotransfection experiments, the coding regions of these constructs were recloned into pRSV-5E1A to generate constructs equivalent to the wild-type E1A vector, as described previously (20a). pRSV-c-*jun* and pRSV-c-*fos* contain the cDNA sequences of human c-*jun* and c-*fos* in the same plasmid background as pRSV-neo and pRSV-5E1A and have been described elsewhere (20a).

The different c-*jun*-CAT constructs contain the indicated c-*jun* promoter sequences in front of the promoterless CAT gene pBLCAT5 (31). Most of these constructs have been described elsewhere (2; the long construct was originally called −1100/+740). The −1600/+170 c-*jun*-CAT construct was obtained by replacing the *Stu*I insert of the −132/+170 construct with the corresponding fragment of −1600/+740. −1600/+170 mTRE was derived similarly from −132/+170 mTRE (2); in the −1600/+740 mTRE clone, the *Sac*I insert of −1600/+170 mTRE is replaced by the corresponding fragment of −1600/+740. collTRE-tata-CAT and c-*jun*TRE-tata-CAT contain one copy of the collTRE and the c-*jun*-TRE, respectively, in front of a TATA box (20a, 31).

RESULTS

Increased c-*jun* mRNA levels in adenovirus-transformed cells. We have reported previously that several mitogen-inducible genes are repressed by adenovirus E1A (21, 32). The repression of one of these genes, the human collagenase gene, involves the inactivation of transcription factor AP-1 (Jun/Fos), which binds to the collTRE (2, 20a). Paradoxically, the level of collTRE-binding factors, although functionally inactive, was found to be elevated in several E1A-expressing cells. This prompted us to analyze the expression levels of the c-*jun* gene that encodes the decisive AP-1 subunit with respect to its binding to the collTRE (6, 20). The cell types examined were HER, BRK, and NRK cells. The expression of the c-*jun* gene was found to be stimulated 5- to 10-fold in both Ad5- and Ad12-transformed cell lines (Fig. 1). The E1A oncogene is responsible for this activation, since NRK cells transformed by E1A alone also showed increased c-*jun* mRNA levels. Strongly elevated levels of c-*jun* mRNA were not observed in several BRK and NRK cell lines transformed by other oncogenes, i.e., the SV40 early region, the polyomavirus early region, or c-H-*ras*(val12) (Fig. 1B and C). Thus, elevated c-*jun* mRNA expression appears to be a general feature of adenovirus-transformed cells and is not common to cells transformed by several other oncogenes.

E1A and mitogens synergistically stimulate c-*jun* and *junB* expression. In NRK cells, the E1A gene of Ad5 stimulated not only the c-*jun* gene but also the related *junB* gene. NRK

cells expressing the E1A gene of Ad12, however, did not show elevated *junB* mRNA levels (Fig. 1C), indicating that *junB*, in contrast to c-*jun*, is differentially regulated by Ad5 and Ad12 E1A. In BRK cells, the regulation of *junB* expression also depends on the adenovirus serotype. In Ad5-transformed BRK cells, *junB* mRNA levels were not altered compared with those in untransformed BRK cells, whereas in Ad12-transformed BRK cells *junB* was even repressed (Fig. 1B). *junB* mRNA could not be detected in any of the HER cells tested (not shown). Thus, the expression of the *junB* gene in adenovirus-transformed cells is determined both by the origin of the progenitor cell and by the transforming-adenovirus serotype.

Since expression of c-*jun* and *junB* is inducible by mitogens (23, 24), we tested whether E1A interferes with the induction by TPA and serum growth factors. Comparison of Ad5 E1-transformed and control NRK cells showed that although E1A already increases basal c-*jun* and *junB* RNA expression, the genes are induced to the same relative extent in both cell lines by serum growth factors (Fig. 2). Similar results were obtained when several other NRK cell lines expressing Ad5 or Ad12 E1A were stimulated with serum or TPA (data not shown). This indicates that E1A and mitogens act synergistically on c-*jun* and *junB* expression.

E1A stimulates c-*jun* promoter activity. To investigate the mechanism by which E1A stimulates c-*jun* expression, we analyzed the effect of E1A on basal and TPA-induced c-*jun* promoter activity in transient expression assays. A c-*jun* promoter construct carrying the human c-*jun* sequences from positions −1600 to +740 fused to the CAT bacterial gene (called −1100/+740 in reference 2) was cotransfected with pRSV-5E1A into HeLa tk^- cells. The latter plasmid expresses the Ad5 E1A proteins from the Rous sarcoma virus promoter. Consistent with our observations in adenovirus-transformed cells, both basal and TPA-induced expression of c-*jun*-CAT was increased more than 10-fold in the presence of Ad5 E1A (Fig. 3). The enhanced expression of c-*jun* in adenovirus-transformed cells, therefore, reflects a direct effect of E1A on c-*jun* transcription, rather than a secondary effect of adenovirus transformation.

In order to define the sequences of the c-*jun* promoter that are involved in the E1A-dependent stimulation, we analyzed the effect of E1A on various c-*jun* promoter fragments (Fig. 4 and 5; Table 1). Our data demonstrate that sequences in both the −1600/−132 and the +170/+740 regions are required for maximal stimulation by E1A: a construct driven by the −132/+170 promoter fragment was not activated significantly by E1A compared with the RSV-CAT control, whereas the −1600/+170 and −132/+740 constructs showed intermediate stimulation by E1A (Table 1). Since the −132/+170 c-*jun* promoter fragment did not confer E1A-dependent inducibility to the reporter gene, the sequences present in this fragment apparently do not play a major role in this E1A effect. These sequences harbor an SP-1- and a CTF-binding element, as well as a sequence that has been shown to bind purified AP-1 in vitro (2). The latter element is termed c-*jun*TRE (2), since it mediates the stimulation by the phorbol ester TPA of the −79/+170 c-*jun* promoter and of a minimal synthetic promoter carrying one copy of the c-*jun*-TRE in front of a TATA element (Table 1). The view that the c-*jun*TRE is not required for the activation by E1A is supported by the fact that neither of these two promoter constructs is significantly stimulated by E1A. It should be noted, however, that mutation of the c-*jun*TRE in the context of the full-length (−1600/+740) c-*jun* promoter does reduce the stimulation by E1A (Table 1, −1600/+740 mTRE). The c-*jun*TRE, although not capable of mediating E1A-dependent stimulation on its own, therefore seems to have a supplementary role in the activation by E1A of the full-length c-*jun* promoter.

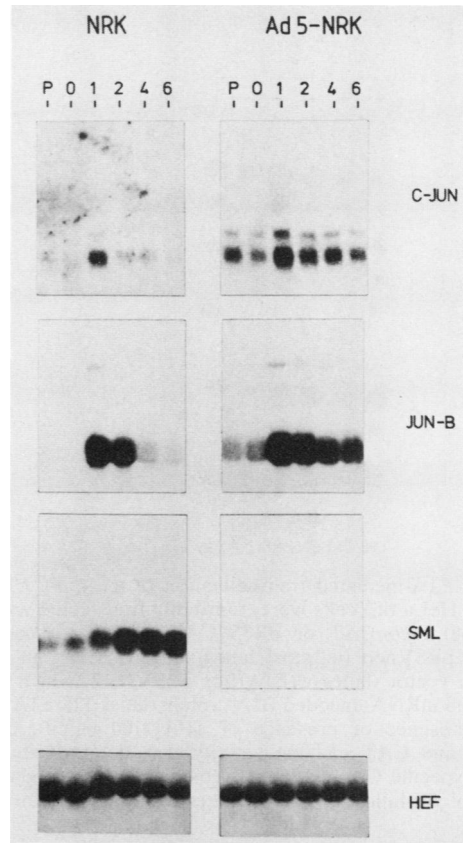

FIG. 2. Induction of c-*jun*, *junB*, and stromelysin mRNAs in NRK cells. Untransformed and adenovirus-transformed (Ad5 E1 clone 1-2) NRK cells were grown to subconfluence and subsequently incubated in medium containing 0.5% FCS for 24 h. Then 10% FCS was added, and cytoplasmic RNA was isolated at the times indicated above the lanes (in hours). The mRNAs were analyzed by Northern blotting and hybridization with the indicated probes. SML, Stromelysin; P, proliferating cells before serum starvation.

Also with respect to activation of the c-*jun* promoter by TPA, the influence of the c-*jun*TRE strongly depends on its neighboring sequences. Inactivation of the c-*jun*TRE abolishes stimulation by TPA of the −79/+170 and c-*jun*TRE-tata promoters (Table 1, compare with −79/+170 mTRE and tata, respectively), whereas this does not affect the TPA inducibility of the −1600/+740 promoter fragment (compare with −1600/+740 mTRE; see Discussion).

In conclusion, the stimulation of c-*jun* promoter activity by E1A is mediated primarily by sequences in the −1600/−132 and +170/+740 regions. The c-*jun*TRE, when presented in the context of the full-length c-*jun* promoter, also contributes to this E1A effect.

E1A differentially affects the activity of AP-1 transcription factors. We have demonstrated earlier that the collTRE is instrumental in the repression of the collagenase gene by E1A. Furthermore, E1A was found to repress synthetic promoters that carry one or more copies of the collTRE (20a). Interestingly, E1A does not inhibit the −79/+170 and c-*jun*TRE-tata promoters, for which the TPA inducibility

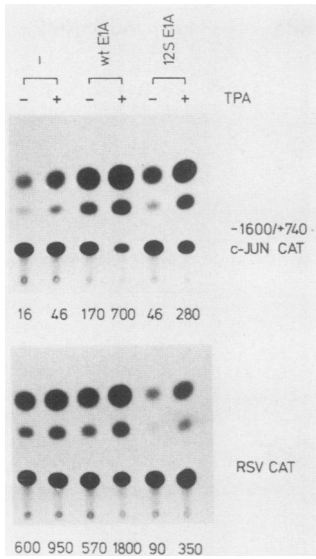

FIG. 3. E1A-mediated transactivation of a c-*jun*-CAT reporter construct. HeLa tk$^-$ cells were transiently transfected with 2 μg of −1600/+740 c-*jun*-CAT or pRSV-CAT and 8μg of one of the following: pRSVneo (leftmost lanes); pRSV-5E1A, an Ad5 E1A expression vector (lanes wtE1A); or pRSVJF12, which expresses only the 12S mRNA-encoded E1A protein (lanes 12S E1A). After 44 h in the absence or presence of TPA (100 ng/ml), cells were harvested and CAT enzyme activity was determined. Numbers represent specific CAT activity (in picomoles of acetylated chloramphenicol per milligram of protein extract per minute).

depends essentially on the c-*jun*TRE (Table 1; Fig. 5). These data indicate that E1A abolishes the TPA-induced activity of the collTRE (TGAGTCA) while not affecting the TPA-induced activity of the c-*jun*TRE (TGACATCA). In addition to TPA, overexpression of Jun protein also leads to stimulation of minimal promoters carrying either the collTRE or the c-*jun*TRE (2, 20a). Since E1A does not affect the stimulation of the c-*jun*TRE by TPA, we were interested whether high levels of Jun could also activate the c-*jun*TRE in the presence of E1A. In Fig. 5, it is shown that the −79/+170 c-*jun* and collTRE-tata promoters display similar inducibility by overexpression of Jun. Consistent with our earlier results (20a), coexpression of E1A abolished the activation of the collTRE. Importantly, the Jun-mediated activation of the c-*jun*TRE was also suppressed by E1A. Thus, E1A represses the activation of the c-*jun*TRE by Jun but does not affect the activation of this element by TPA. We hypothesize that the activation of the c-*jun*TRE by TPA is

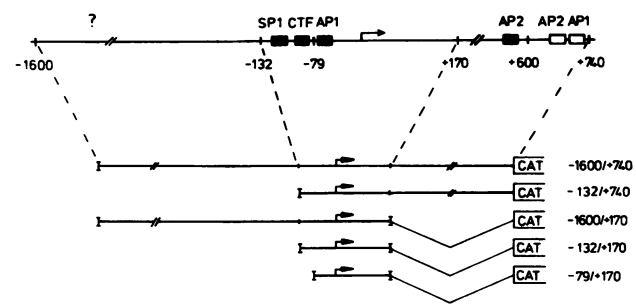

FIG. 4. Map of the c-*jun* promoter region, showing the locations of several transcription factor binding sites (2) and the c-*jun* promoter fragments present in CAT constructs.

mediated by an AP-1-related factor different from AP-1 (Jun/Fos) and that the activity of this factor is not abolished by E1A (see Discussion).

The fact that E1A inhibits the Jun-dependent stimulation of the c-*jun*TRE predicts that the combination of Jun and E1A should not act synergistically at the full-length (−1600/+740) c-*jun* promoter. Interestingly, Jun was found to even inhibit stimulation of this promoter by E1A (Fig. 5; note that Fos does not elicit this effect). Moreover, also in the absence of E1A, high levels of Jun did not lead to an increase in c-*jun* promoter activity. Together these data indicate that Jun has an inhibitory effect on one or more sequences within the c-*jun* promoter, thereby counteracting its own stimulatory effect on the c-*jun*TRE, as well as the E1A-mediated activation. Since the −79/+170 promoter fragment was stimulated, rather than inhibited, by high levels of Jun (Fig. 5), the sequences mediating the Jun inhibitory effect must be located outside this region. The negative autoregulation by Jun may, in fact, reflect the transient character of the stimulation of c-*jun* gene expression by mitogens.

Requirement of the transforming domain CR1. The adenovirus E1A gene contains three conserved regions (CR1, CR2, and CR3; Fig. 6A), which have been shown to play a role in either the inhibition (CR1 and CR2) or the stimulation (CR3) of gene expression (17, 18, 26). To investigate which of these E1A-domains is involved in the elevation of c-*jun* expression, we examined a series of mutant E1A constructs for their ability to stimulate c-*jun* promoter activity in a transient expression assay. These experiments indicated that mutations in CR2 and CR3 do not affect the stimulation of c-*jun* promoter activity, whereas mutation of CR1 abolishes this E1A effect (Fig. 3; Table 2). CR1 was also shown to be required for the activation of c-*jun* and *junB* expression in a panel of NRK cells stably expressing the mutant E1A genes (Fig. 6). The activation of these genes by E1A, therefore, differs from the E1A-mediated transactivation of various viral and cellular genes, for which CR3 alone is responsible (10, 16, 18, 26). In conclusion, the requirement for CR1, a domain essential for transformation, links the activation of c-*jun* and *junB* expression to the E1A-driven transformation process.

DISCUSSION

In this paper, we show that the adenovirus E1A oncogene can constitutively enhance the expression of c-*jun* and *junB*, genes that encode components of the AP-1 family of transcription factors (6, 20). In the same adenovirus-transformed cells the expression of *junD*, another member of the *jun* family (12, 22), and of c-*fos* is unaltered (33; H. van Dam, unpublished results), indicating that E1A differentially affects the expression levels of various transcription factor-encoding genes. Various other oncogene products (e.g., Ras proteins) have been shown to stimulate transcription through AP-1 binding sites (28, 37) or, in the case of SV40 transformation, to elevate AP-1 binding activity (13). Furthermore, transiently elevated c-Ha-*ras* expression can stimulate c-*fos*, c-*jun*, and *junB* expression (27–29). However, in cell lines stably transformed by *ras* or SV40, we and others have not been able to detect strongly elevated c-*jun* expression (Fig. 1) (29). Therefore, the constitutive activation of c-*jun* expression appears to be a common feature of cells transformed by E1A, whereas transformation by several other oncogenes does not generally lead to increased c-*jun* levels.

The elevation of c-*jun* expression is due to stimulation of the c-*jun* promoter activity by the E1A proteins. Maximal

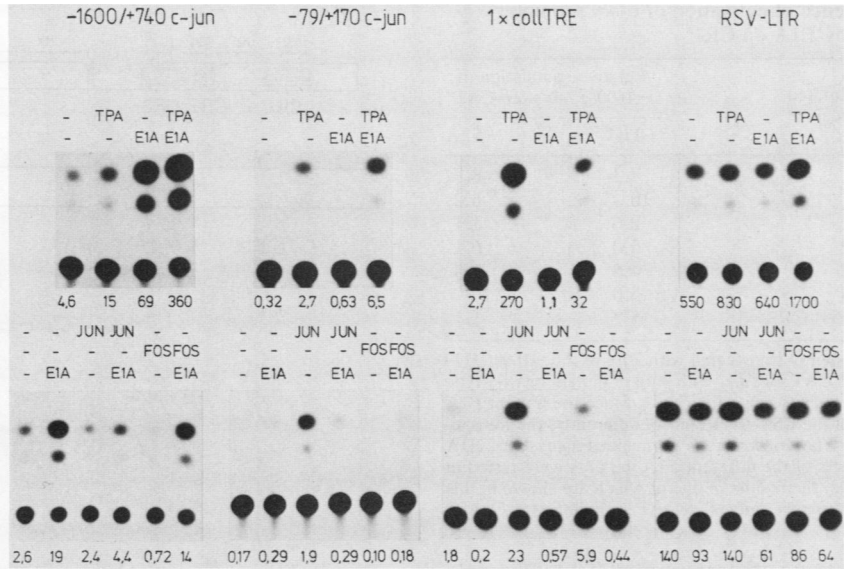

FIG. 5. Effects of TPA and Jun protein on the c-*jun* promoter in the presence of E1A. HeLa tk$^-$ cells were transiently transfected with 2 μg of the indicated c-*jun*-CAT constructs, pcollTRE-tata-CAT, or pRSV-CAT together with 6 μg each of pRSV-5E1A, pRSV-c-*jun*, pRSV-c-*fos*, and/or pRSVneo (−). After transfection, the cells were grown in medium containing 10% FCS in the absence or presence of TPA (upper panels) or in medium containing 0.5% FCS (lower panels). Forty hours later, cells were harvested and CAT enzyme activity was determined. Numbers represent specific CAT activity (in picomoles of acetylated chloramphenicol per milligram of protein extract per minute).

stimulation of the c-*jun* promoter by E1A requires sequences both upstream and downstream of the −132/+170 region (Table 1). The −1600/+170 and −132/+740 c-*jun*-CAT constructs display suboptimal stimulation by E1A, indicating that the −1600/−132 and the +170/+740 regions contain E1A-inducible elements that can act in a cooperative manner. Mutation of the TPA-responsive element between positions −72 and −63 (c-*jun*TRE) also reduced the stimulation of the −1600/+740 c-*jun* promoter by E1A, indicating that this sequence plays an additional role in the stimulation by E1A. However, the activity of the c-*jun*TRE, when present in a small fragment (−79/+170) of the c-*jun* promoter or in a minimal synthetic promoter, was neither stimulated nor repressed by E1A (Table 1). Thus, the c-*jun*TRE functions differently in the contexts of the full-length c-*jun* promoter and of smaller promoter fragments.

Our data, furthermore, reveal that after stimulation by TPA the c-*jun*TRE, in contrast to the collTRE, is not inhibited by E1A. Since earlier studies have shown that E1A represses the collTRE by inhibiting the activity of the transcription factor AP-1 (Jun/Fos), we tentatively conclude that TPA induces a different member of the AP-1 family to stimulate gene expression via the c-*jun*TRE. Although the transcription factor AP-1 was originally described as a heterodimeric complex of Jun and Fos (8, 25), a number of Jun- and Fos-related proteins have been described which function as constituents of AP-1-like transcription factors (for a review, see P. K. Vogt and T. J. Bos, Adv. Cancer Res., in press). Moreover, it has recently been shown that Jun can also form heterodimers with members of the CREB/ATF family (4, 15). Consistent with the observation that the collTRE and the c-*jun*TRE are differentially regulated by E1A, we now have evidence that the protein complexes binding to these elements display distinct properties (B. Stein et al., unpublished data).

As mentioned above, the role of the c-*jun*TRE in the stimulation of promoter activity by E1A strongly depends on the context in which this element is presented. A similar conclusion can be drawn with respect to the stimulation by TPA: mutation of the c-*jun*TRE abolishes the TPA inducibility of the −79/+170 c-*jun* promoter fragment, whereas the same mutation does not affect TPA induction of the full-length c-*jun* promoter (Table 1). This implies that, in addition

TABLE 1. Stimulation of c-*jun* promoter mutants by E1A, TPA and Jun[a]

Construct	E1A	CAT activity (pmol/mg/min)		Stimulation factor with:			
		−TPA	+TPA	TPA	E1A −TPA	E1A +TPA	Jun
−1600/+740	−	10	34	3.4			1.2
	+	120	720		12	21	
−132/+740	−	2.1	6.9	3.3			2.5
	+	8.0	50		3.8	7.2	
−1600/+170	−	10	22	2.2			1.1
	+	39	200		3.9	9.1	
−132/+170	−	1.7	3.9	2.3			2.0
	+	2.5	11		1.5	2.8	
−79/+170	−	0.80	7.0	8.7			12
	+	1.3	13		1.6	1.9	
−1600/+740 mTRE	−	5.8	22	3.8			NT[b]
	+	39	370		6.8	17	
−79/+170 mTRE	−	0.54	0.86	1.6			NT
	+	1.1	2.4		2.0	2.8	
c-*jun*TRE-tata	−	0.32	2.2	6.9			NT
	+	0.45	4.0		1.4	1.8	
tata	−	0.34	0.30	0.9			NT
	+	0.37	0.46		1.1	1.5	
AdE3	−	3.8	9.5	2.5			NT
	+	120	1,400		32	150	
RSV-LTR	−	1,300	2,000	1.5			2.3
	+	1,500	4,800		1.2	2.4	

[a] HeLa tk$^-$ cells were transiently transfected with 2 μg of the indicated c-*jun*-CAT constructs or pRSV-CAT together with 8 μg of either pRSV-5E1A or pRSVneo (−), as described for Fig. 3. An adenovirus E3 promoter CAT gene was tested as a positive control for E1A transactivation (38). Activation by Jun protein was tested as described for Fig. 5. Data represent the average of three independent experiments.

[b] NT, Not tested.

TABLE 2. Dependence of activation of c-*jun* promoter by E1A on CR1[a]

Construct	Mutated region	Relative stimulation of −1600/+740 c-*jun*-CAT	
		−TPA	+TPA
wtE1A		7.3	8.7
13S		10	6.2
G5/3	CR1	0.6	1.1
GCX	CR2	5.0	6.1
G3/2	CR2	4.6	7.4
JF12 (12S)	CR3	5.0	10
G5/3-JF12	CR1 + CR3	1.2	1.1

[a] HeLa tk$^-$ cells were transiently transfected with 2 μg of the −1600/+740 c-*jun*-CAT construct or pRSV-CAT together with 8 μg of mutant or wild-type Ad5 E1A expression vectors, as described for Fig. 3. Since the mutant E1A vectors affected the activity of the RSV-CAT control differently, the transactivation of c-*jun*-CAT by the E1A constructs is normalized against the E1A effect on RSV-CAT. The effects of the different E1A mutants were tested in three independent experiments, the results of one of which are shown in this table. The outcomes of all three experiments were very similar.

to the c-*jun*TRE, at least one other important TPA-inducible element is present in the c-*jun* promoter. Indeed, a second TPA-inducible element has recently been delimited (Stein et al., unpublished data).

The regulation of the c-*jun* gene by its own gene product (Jun) also involves multiple regulatory elements. It has been reported previously that overexpression of Jun leads to stimulation, through the c-*jun*TRE, of the −79/+170 c-*jun*-CAT gene as well as of constructs containing longer c-*jun* promoter fragments (2). In our experiments, however, only the −79/+170 construct was strongly stimulated by high levels of Jun, whereas the −1600/+740 and −1600/+170 c-*jun* promoter fragments were not activated (Table 1; Fig. 5). Since our experiments were performed with HeLa tk$^-$ cells, whereas Angel and co-workers used F9 teratocarcinoma cells in their study, this discrepancy probably reflects cell-type-specific features, most likely the fact that HeLa tk$^-$ cells contain considerable basal AP-1 activity, whereas F9 cells display no such activity at all (8). A possible explanation is that Jun, in addition to the stimulatory effect on the c-*jun*TRE, has an equally strong repressive effect on sequences located further upstream in the c-*jun* promoter. This model is, in fact, supported by the observation that high levels of Jun counteract the stimulation of the −1600/+740 c-*jun*-CAT gene by E1A (Fig. 5). We hypothesize that the inhibitory action of Jun on the c-*jun* promoter represents a negative feedback, possibly the same feedback that causes this promoter to be only transiently induced after stimulation of cells by mitogens (24, 36; Vogt and Bos, in press). Whether this feedback mechanism is a direct effect of Jun or requires Jun-dependent regulation of other genes remains to be determined. Irrespective of the mechanism, it is important to note that this function of Jun, in contrast to its transactivation function at the collTRE and the c-*jun*TRE, apparently is not inhibited by E1A. This suggests that E1A does not abolish all activities of Jun, implying a functional relevance for the increase in c-*jun* gene expression in adenovirus-transformed cells.

The stimulation of the c-*jun* and *junB* genes is a function of the transforming domain in CR1, indicating that it is likely to be part of the transformation mechanism. Furthermore, the nononcogenic serotype Ad5 and the highly oncogenic Ad12 regulate *junB* expression differently, suggesting that modulation of transcription factor levels may influence the oncogenic properties of adenovirus-transformed cells. The inactivation of the transcription factor AP-1 (Jun/Fos) is also linked to the process of adenovirus transformation through its dependence on CR1 (20a). Our data furthermore illustrate that the gene-modulatory function of E1A is selective. First, we show that E1A does not inhibit all AP-1 family members, as is illustrated by the observation that the activity of the

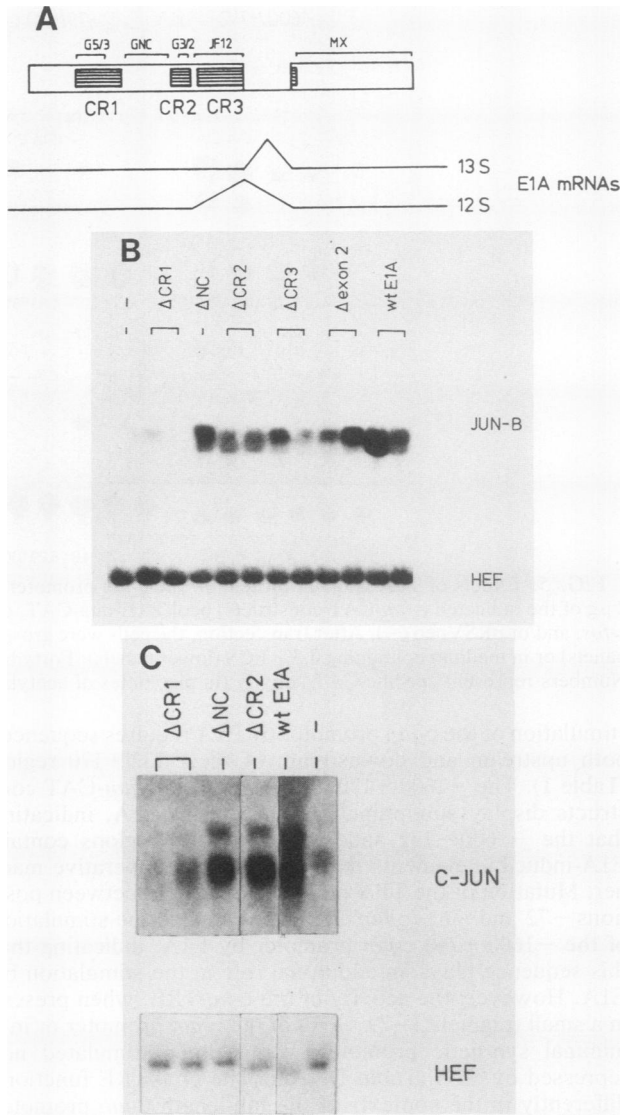

FIG. 6. Effects of mutations in Ad5 E1A on the E1A-mediated activation of *junB* and c-*jun* mRNA expression. (A) Schematic representation of the Ad5 E1A gene and the deletions present in the different mutant constructs. The 13S cDNA coding sequence (▭) and the conserved regions within the E1A gene (amino acids 41 to 73 [CR1], 121 to 136 [CR2], and 143 to 188 [CR3]) (▤) are shown. The following amino acid residues were deleted from the various mutants: G5/3, 38 to 65; GNC, 86 to 120; G3/2, 125 to 133; JF12, 140 to 185; and MX, 185 to 289. (B) RNA was isolated from untransformed NRK cells (lane −) and NRK cell lines expressing wild-type or mutant E1A genes (34) and analyzed by Northern blotting. The following cell lines were examined: G5/3 clones 4-1 and 4-3 (lanes ΔCR1), GNC clone A (lane ΔNC), G3/2 clones 1-1 and 2-1 (lanes ΔCR2), JF12 clones 5-2 and 1-1 (lanes ΔCR3), MX clones 2-2 and 1-3 (lanes Δexon 2), and wtE1 clone 1-2 and wtE1A clone 1-1 (lanes wtE1A). (C) Cell clones examined were G5/3 clones 4-1 and 4-3 (lanes ΔCR1), GNC clone A (lane ΔNC), G3/2 clone 2-1 (lane ΔCR2), and wtE1 clone 1-2 (lane wtE1A). Lane −, Untransformed cells.

AP-1-related factor that binds to the c-*jun*TRE is not repressed. Second, we show that E1A, although it suppresses the Jun-dependent transactivation of the c-*jun*TRE and collTRE, does not abolish all activities of Jun. Since Jun can dimerize with a variety of other transcription factor proteins belonging either to the AP-1 family or to the CREB/ATF family (4, 15; Vogt and Bos, in press), it is conceivable that E1A can inhibit activities of some Jun-containing transcription factors, whereas the activities of others are not affected or even stimulated.

In conclusion, E1A influences the repertoire of (AP-1-related) transcription factors both by altering the synthesis of their constituents and by selectively affecting their functions. Since several genes that encode constituents of AP-1 and AP-1-related factors have been found to display transforming properties (36; Vogt and Bos, in press), it is tempting to speculate that modulation of AP-1 function results in altered expression of cellular genes directly involved in transformation.

ACKNOWLEDGMENTS

We thank R. Bernards, D. Nathans, and M. Yaniv for giving us the c-*jun*, *junB*, and *junD* probes; P. Angel and M. Karin for several of the c-*jun* promoter constructs; and N. Jones for the AdE3-CAT gene. Furthermore, we thank D. Roelen, H. T. M. Timmers, and K. Timmers for contributions to some of the experiments and A. Zantema, A. G. Jochemsen, and H. van Ormondt for help in preparing the manuscript.

This work was supported by the Netherlands Organization for Scientific Research (NWO) through the Foundation for Medical and Health Research (MEDIGON) and the Foundation of Chemical Research in the Netherlands (SON).

LITERATURE CITED

1. **Angel, P., I. Baumann, B. Stein, H. Delius, H. J. Rahmsdorf, and P. Herrlich.** 1987. 12-*O*-Tetradecanoyl-phorbol-13-acetate induction of the human collagenase gene is mediated by an inducible enhancer element located in the 5'-flanking region. Mol. Cell. Biol. **7**:2256–2266.
2. **Angel, P., K. Hattori, T. Smeal, and M. Karin.** 1988. The jun proto-oncogene is positively autoregulated by its product, Jun/AP-1. Cell **55**:875–885.
3. **Angel, P., M. Imagawa, R. Chiu, B. Stein, R. J. Imbra, H. J. Rahmsdorf, C. Jonat, P. Herrlich, and K. Karin.** 1987. Phorbol ester-inducible genes contain a common cis element recognized by a TPA-modulated trans-acting factor. Cell **49**:729–739.
4. **Benbrook, D. M., and N. C. Jones.** 1990. Heterodimer formation between CREB and JUN proteins. Oncogene **5**:295–302.
5. **Berk, A. J.** 1986. Adenovirus promoters and E1A transactivation. Annu. Rev. Genet. **20**:45–79.
6. **Bohmann, D., T. J. Bos, A. Admon, T. Nishimura, P. K. Vogt, and R. Tjian.** 1987. Human proto-oncogene c-jun encodes a DNA binding protein with structural and functional properties of transcription factor AP-1. Science **238**:1386–1392.
7. **Borrelli, E., R. Hen, and P. Chambon.** 1984. Adenovirus 2 E1A products repress enhancer-induced stimulation of transcription. Nature (London) **312**:608–612.
8. **Chiu, R., W. J. Boyle, J. Meek, T. Smeal, T. Hunter, and M. Karin.** 1988. The c-Fos protein interacts with c-Jun/AP-1 to stimulate transcription of AP-1 responsive genes. Cell **54**:541–552.
9. **Egan, C., T. N. Jelsma, J. A. Howe, S. T. Bayley, B. Ferguson, and P. E. Branton.** 1988. Mapping of cellular protein-binding sites on the products of early-region 1A of human adenovirus type 5. Mol. Cell. Biol. **8**:3955–3959.
10. **Green, M., P. M. Loewenstein, R. Pusztai, and J. S. Symington.** 1988. An adenovirus E1A protein domain activates transcription in vivo and in vitro in the absence of protein synthesis. Cell **53**:921–926.
11. **Hen, R., E. Borrelli, and P. Chambon.** 1985. Repression of the immunoglobulin heavy chain enhancer by the adenovirus-2 E1A products. Science **230**:1391–1394.
12. **Hirai, S.-I., R.-P. Ryseck, F. Mechta, R. Bravo, and M. Yaniv.** 1989. Characterization of *junD*: a new member of the *jun* proto-oncogene family. EMBO J. **8**:1433–1439.
13. **Hirai, S.-I., and M. Yaniv.** 1988. Constitutive synthesis of activator protein 1 transcription factor after viral transformation of mouse fibroblasts. Proc. Natl. Acad. Sci. USA **85**:3401–3405.
14. **Jelsma, T. N., J. A. Howe, J. S. Mymryk, C. M. Evelgh, N. F. A. Cunniff, and S. T. Bayley.** 1989. Sequences in E1A proteins of human adenovirus 5 required for cell transformation, repression of a transcriptional enhancer, and induction of proliferating cell nuclear antigen. Virology **171**:120–130.
15. **Jones, N. C., P. W. J. Rigby, and E. B. Ziff.** 1988. *trans*-acting protein factors and the regulation of eukaryotic transcription: lessons from studies on DNA tumor viruses. Genes Dev. **2**:267–281.
16. **Lillie, J. W., and M. R. Green.** 1989. Transcription activation by the adenovirus E1A protein. Nature (London) **338**:39–44.
17. **Lillie, J. W., M. Green, and M. R. Green.** 1986. An adenovirus E1A protein region required for transformation and transcriptional repression. Cell **46**:1043–1051.
18. **Lillie, J. W., P. M. Loewenstein, M. R. Green, and M. Green.** 1987. Functional domains of adenovirus type 5 E1A proteins. Cell **50**:1091–1100.
19. **Moran, E., B. Zerler, T. M. Harrison, and M. B. Mathews.** 1986. Identification of separate domains in the adenovirus E1A gene for immortalization activity and the activation of virus early genes. Mol. Cell. Biol. **6**:3470–3480.
20. **Nakabeppu, Y., K. Ryder, and D. Nathans.** 1988. DNA binding activities of three murine jun proteins: stimulation by fos. Cell **55**:907–915.

20a. **Offringa, R., S. Gebel, H. van Dam, M. Timmers, A. Smits, R. Zwart, B. Stein, J.-L. Bos, A. J. van der Eb, and P. Herrlich.** 1990. A novel function of the transforming domain of E1A: Repression of AP-1 activity. Cell **62**:527–538.

21. **Offringa, R., A. M. M. Smits, A. Houweling, J. L. Bos, and A. J. Van der Eb.** 1988. Similar effects of adenovirus E1A and glucocorticoid hormones on the expression of the metalloprotease stromelysin. Nucleic Acids Res. **16**:10973–10984.
22. **Ryder, K., A. Lanahan, E. Perez-Albuerne, and D. Nathans.** 1989. *Jun-D*. A third member of the *Jun* gene family. Proc. Natl. Acad. Sci. USA **86**:1500–1503.
23. **Ryder, K., L. F. Lau, and D. Nathans.** 1988. A gene activated by growth factors is related to the oncogene v-*jun*. Proc. Natl. Acad. Sci. USA **85**:1487–1491.
24. **Ryseck, R.-P., S. I. Hirai, M. Yaniv, and R. Bravo.** 1988. Transcriptional activation of c-*jun* during the G_0/G_1 transition in mouse fibroblasts. Nature (London) **334**:535–537.
25. **Sassone-Corsi, P., W. W. Lamph, M. Kamps, and I. M. Verma.** 1988. *fos*-associated cellular p39 is related to nuclear transcription factor AP-1. Cell **54**:553–560.
26. **Schneider, J. F., F. Fisher, C. R. Goding, and N. C. Jones.** 1987. Mutational analysis of the adenovirus E1A gene: the role of transcriptional regulation in transformation. EMBO J. **6**:2053–2060.
27. **Schönthal, A., S. Gebel, B. Stein, H. Ponta, H. J. Rahmsdorf, and P. Herrlich.** 1988. Nuclear oncoproteins determine the genetic program in response to external stimuli. Cold Spring Harbor Symp. Quant. Biol. **53**:779–787.
28. **Schönthal, A., P. Herrlich, H. J. Rahmsdorf, and H. Ponta.** 1988. Requirement for fos gene expression in the transcriptional activation of collagenase by other oncogenes and phorbol esters. Cell **54**:325–334.
29. **Sistonen, L., E. Holtta, T. P. Makela, J. Keski-Oja, and K. Alitalo.** 1989. The cellular response to induction of the $p21^{c\text{-}Ha\text{-}ras}$ oncoprotein includes stimulation of *jun* gene expression. EMBO J. **8**:815–822.
30. **Stabel, S., P. Argos, and L. Philipson.** 1985. The release of growth arrest by microinjection of adenovirus E1A. EMBO J. **4**:2329–2336.
31. **Stein, B., H. J. Rahmsdorf, A. Steffen, M. Litfin, and P. Herrlich.** 1989. UV-induced DNA damage is an intermediate in

UV-induced expression of human immunodeficiency virus type 1, collagenase, c-*fos*, and metallothionein. Mol. Cell. Biol. **9**:5169–5181.

32. **Timmers, H. T. M., D. De Wit, J. L. Bos, and A. J. Van der Eb.** 1988. E1A products of adenoviruses reduce the expression of proliferation-associated genes. Oncogene Res. **3**:67–76.
33. **Timmers, H. T. M., H. Van Dam, G. J. Pronk, J. L. Bos, and A. J. Van der Eb.** 1989. Adenovirus E1A represses transcription of the cellular JE gene. J. Virol. **63**:1470–1473.
34. **Van Dam, H., R. Offringa, A. M. M. Smits, J. L. Bos, N. C. Jones, and A. J. Van der Eb.** 1989. The repression of the growth factor-inducible genes JE, c-*myc* and stromelysin by adenovirus E1A is mediated by conserved region 1. Oncogene **4**:1207–1212.
35. **Van der Lubbe, J. L. M., P. J. Abrahams, C. M. Van Drunen, and A. J. Van der Eb.** 1986. Enhanced induction of SV40 replication from transformed rat cells by fusion with UV-irradiated normal and repair-deficient human fibroblasts. Mutat. Res. **165**:47–56.
36. **Vogt, P. K., and T. J. Bos.** 1989. The oncogene *jun* and nuclear signalling. Trends Biochem. Sci. **14**:172–175.
37. **Wasylyk, C., J. L. Imler, and B. Wasylyk.** 1988. Transforming but not immortalizing oncogenes activate the transcription factor PEA1. EMBO J. **7**:2475–2483.
38. **Weeks, D. L., and N. C. Jones.** 1983. E1a control of gene expression is mediated by sequences 5′ to the transcriptional starts of the early genes. Mol. Cell. Biol. **3**:1222–1234.
39. **Whyte, P., K. J. Buchkovich, J. M. Horowitz, S. H. Friend, M. Raybuck, R. A. Weinberg, and E. Harlow.** 1988. Association between an oncogene and an anti-oncogene: the adenovirus E1A proteins bind to the retinoblastoma gene product. Nature (London) **334**:124–129.
40. **Whyte, P., N. M. Williamson, and E. Harlow.** 1989. Cellular targets for transformation by the adenovirus E1A proteins. Cell **56**:67–75.