*Proc. Natl. Acad. Sci. USA*
Vol. 83, pp. 3213–3217, May 1986
Biochemistry

# Transcriptional activation encoded by the v-*fos* gene

[*fos* oncogene/*trans*-activation/$\alpha_1$(III) collagen gene/long terminal repeat of Rous sarcoma virus]

CHIAKI SETOYAMA*, RODOLFO FRUNZIO†, GENE LIAU‡, MARIA MUDRYJ, AND BENOIT DE CROMBRUGGHE§

Laboratory of Molecular Biology, Building 37, Room 2D20, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

*Communicated by Maxine Singer, January 7, 1986*

**ABSTRACT** **We present evidence that the *fos* oncogene encodes a transcriptional *trans*-activation function. *trans*-activation was assayed by cotransfection into NIH 3T3 mouse fibroblasts of v-*fos* DNA containing plasmids together with a plasmid containing a test promoter. Three v-*fos* DNAs were used: (*i*) pFBR-1, a plasmid containing the FBR proviral sequences; (*ii*) pFBJ-2, a plasmid harboring the FBJ proviral sequences; (*iii*) pMF-J, a plasmid containing the FBJ *fos* sequences linked to a mouse metallothionein promoter. Each of the three v-*fos* DNA plasmids stimulated the expression of a cotransfected chimeric gene consisting of a promoter segment of the mouse $\alpha_1$(III) collagen gene linked to the gene for chloramphenicol transacetylase. In similar experiments the v-*fos* gene also stimulated the long terminal repeat promoter of Rous sarcoma virus (RSV) but neither the early promoter of simian virus 40 nor the $\beta$-actin promoter. Evidence that the *trans*-activation function is specified by the v-*fos* coding sequences comes from the fact that a frameshift mutation in the v-*fos* coding sequence inhibits the *trans*-activation. Two mutations that map around nucleotide −100 in the RSV promoter do not respond to cotransfection with v-*fos*, whereas other mutations respond like the wild-type RSV promoter. These experiments suggest that the v-*fos* gene either encodes or induces an activator of transcription that recognizes specific sequences in promoters.**

Both the v-*fos* gene and the c-*fos* gene, if linked to a strong promoter, are capable of transforming fibroblasts (1). Retroviruses harboring the v-*fos* genomic sequences as well as cells transformed by the v-*fos* proviral sequences induce tumors in rodents (2–5). The products of both the c-*fos* and v-*fos* genes are localized in the nucleus (6).

Arguments have been presented which favor the view that c-*fos* may be involved in the differentiation of certain cells. Indeed, both c-*fos* and its transcript show a rapid induction during differentiation of monocytic cells into macrophages (7, 8). There is also a rapid increase in c-*fos* after treatment of pheochromocytoma cells with nerve growth factor (9). Furthermore, transfection of a plasmid containing the c-*fos* gene into embryonal carcinoma cells leads to a certain degree of differentiation of these cells (10). In other experiments, it was shown that the addition of platelet-derived growth factor or serum to quiescent fibroblasts induces a rapid but transient elevation of c-*fos* and of its transcript (11–14), implying some role in the growth control of these cells.

The mechanisms by which v-*fos* induces tumors or c-*fos* may regulate growth and differentiation are unknown. We show here that the v-*fos* gene encodes a transcriptional *trans*-activation function. This function was assayed in transient expression experiments of NIH 3T3 cells after cotransfection with both a plasmid containing v-*fos* and a plasmid containing a test promoter. Two promoters that are stimulated in response to v-*fos* are the promoters of the mouse $\alpha_1$(III) collagen gene and the long terminal repeat (LTR) promoter of Rous sarcoma virus (RSV). Two RSV promoter mutations that map between nucleotides −98 and −113 do not respond to the *trans*-activation mediated by v-*fos*.

## MATERIALS AND METHODS

**Cell Cultures, DNA Transfections, Chloramphenicol Transacetylase (CAT) Assays, and RNA Analysis.** NIH 3T3 mouse fibroblasts were cultured in Dulbecco-modified Eagle's medium supplemented with 10% calf serum. Transfections with recombinant DNAs were carried out by using the calcium phosphate precipitation method. For transient assays, NIH 3T3 cells were seeded at a density of 4–5 × $10^5$ cells per 100-mm dish and 24 hr later were transfected with 5–20 $\mu$g of recombinant DNA. After 48 hr, the cells were harvested for assay of CAT or analysis of RNA. CAT assays were performed as described (15); activities were expressed as percent conversion of [$^{14}$C]chloramphenicol to the acetylated forms. Total RNAs were isolated as described (16). Primer extension experiments were done with a synthetic oligonucleotide complementary to CAT RNA. Conditions of hybridization and primer extension were as described previously (17).

**Plasmids.** Plasmid pPrC3-1 (Fig. 1*b*) is a derivative of pAZ1009 (21) in which the mouse $\alpha_2$(I) collagen promoter fragment has been replaced by a mouse $\alpha_1$(III) collagen gene fragment containing the promoter of this gene between 2.3 kilobases (kb) 5′ of the start site of transcription to position +16 3′ of this site. The $\alpha_1$(III) collagen DNA segment was taken from the recombinant phage PMC3A-5 (22). This plasmid also contains, 3′ to the CAT gene, the SV40 small tumor antigen splicing sites, the early region polyadenylylation site, and a segment containing the SV40 enhancer.

Plasmid PrC3-104 was obtained from pPrC3-1 by deleting a *Bam*HI fragment containing the SV40 enhancer sequences.

Plasmid MF-J (Fig. 1*a*) was constructed by using plasmid pdMMTneo, a gift of P. M. Howley (23). This plasmid contains the promoter region of the mouse metallothionein I gene. A 2.0-kb *Bgl* II/*Hpa* I fragment containing the *neo* gene and SV40 DNA sequences was replaced by a 1.5-kb *Fnu*dII–*Pvu* II fragment isolated from pFBJ-2 DNA, a generous gift of T. Curran (19). This fragment contains sequences encoding the p55 fos protein. The noncomplementary ends were converted to blunt ends by using the *Escherichia coli*

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: LTR, long terminal repeat; RSV, Rous sarcoma virus; CAT, chloramphenicol transacetylase; SV40, simian virus 40; kb, kilobase(s), bp, base pair(s).

*Present address: Department of Biochemistry, Kumamoto University School of Medicine, 2-2-1 Honjo, Kumamoto 860, Japan.
†Present address: Department of Cellular and Molecular Biology and Pathology, II Facolto Di Medicina, Universita Di Napoli, Via S. Pansani 5, 80131 Naples, Italy.
‡Present address: Revlon Biotechnology Research Center, 2501 Research Boulevard, Rockville, MD 20850.
§To whom reprint requests should be addressed.

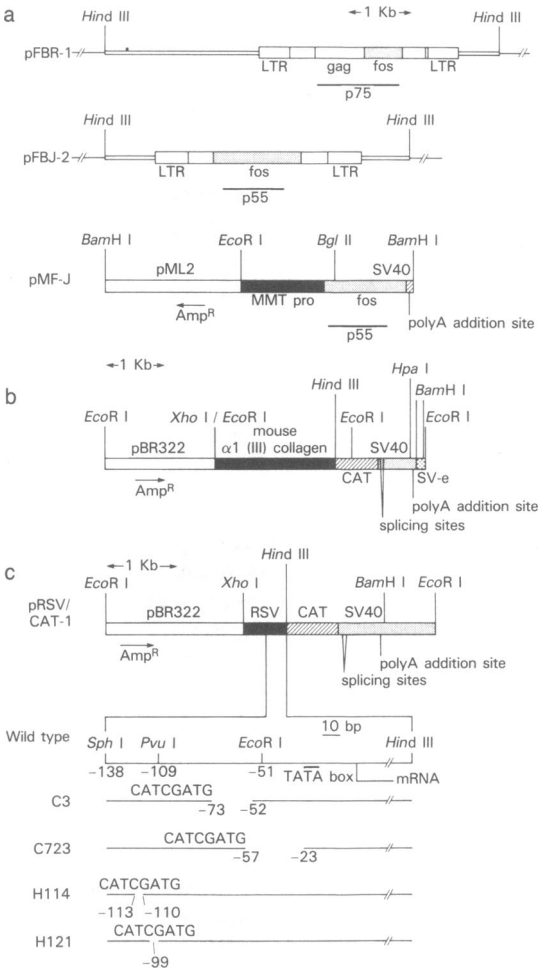


FIG. 1. Schematic representations of plasmids. (*a*) v-*fos*-containing plasmids. Plasmid pFBR-1 represents a mouse DNA segment containing the FBR proviral sequences in the *Hin*dIII site of pBR322 (18). Plasmid pFBJ-2 represents a mouse DNA segment containing the FBJ proviral sequences similarly inserted into the *Hin*dIII site of pBR322 (19). pMF-J is a plasmid in which the mouse metallothionein I promoter is linked to the FBJ-*fos* sequence. It is composed of pML2 sequences (pML2 is a derivative of pBR322 containing the ampicillin gene and the replication origin of pBR322); a 1.6-kb *Eco*RI–*Bgl* II fragment containing the mouse metallothionein I promoter (MMT pro); a 1.5-kb *Fnu*dII–*Pvu* II fragment containing the v-*fos* coding sequences isolated from pFBJ-2 DNA, and a simian virus 40 (SV40) segment that spans the early region polyadenylylation site. (*b*) pPrC3-1 is a plasmid in which the mouse $\alpha_1$(III) collagen promoter is fused to the gene for CAT. The SV40 segment to the right of the CAT gene contains the splicing sites of the small tumor antigen gene and the early region polyadenylylation sites; SV-e is a short SV40 fragment containing one intact copy and part of one copy of the 72-base-pair (bp) repeat sequence. (*c*) pRSVCAT-1 is a derivative of pSV2-CAT (15) in which a ≈640-bp *Pst* I fragment of pSR1 (20) replaces the SV40 promoter fragment. pSR1 is a plasmid that contains the 3′ LTR of the Schmidt–Ruppin strain of RSV inserted into the *Pst* I site of pBR322. The magnified region is the segment between the *Sph* I site at −138 preceding the start of transcription to the *Hin*dIII site. End points of deletions (C3, C723, H114, H121) are indicated for each mutant as well as the substituting sequence. Construction of pRSVCAT-1 and the deletion-substitution mutants will be described in detail elsewhere.

Klenow fragment of DNA polymerase I, before circularization with T4 DNA ligase.

Frameshift mutant MF-J-B was constructed by cleaving MF-J DNA with *Bgl* II, filling out the resulting staggered ends with Klenow fragment, and religating the blunt ends with T4 DNA ligase.

A detailed description of pRSVCAT-1 and mutants C3, C723, H114, and H121 will be reported elsewhere (R.F., unpublished data). Plasmid p-SE-90 contains the 87-bp *Sph* I–*Eco*RI fragment of the RSV (Schmidt–Ruppin strain) LTR inserted into the *Eco*RI and *Sph* I sites of pBR322.

## RESULTS

We wanted to determine whether the fos protein has a *trans*-activation function that might help explain its role in differentiation, transformation, or both. Since we had observed a considerable increase in the expression of the type III collagen gene in v-*fos*-transformed cells (C.S., unpublished data), plasmid pPrC3-1 was constructed in which the 5′ flanking sequences of the mouse $\alpha_1$(III) collagen gene (22) were fused to the bacterial CAT gene (Fig. 1*b*). This plasmid was used in cotransfection experiments with three different plasmids containing v-*fos* sequences: (*i*) pFBR-1, a plasmid containing the FBR proviral sequences (18); (*ii*) pFBJ-2, a plasmid containing the FBJ proviral sequences (19); (*iii*) pMF-J, a plasmid containing the FBJ *fos* sequences linked to a mouse metallothionein promoter. These plasmids are schematically shown in Fig. 1*a*. Cotransfection of NIH 3T3 cells with the DNA of pPrC3-1 and the plasmid containing the FBR genomic sequences (pFBR-1) results in a 5- to 10-fold increase in the levels of CAT 48 hr after transfection (Fig. 2*a*, lane 2). In the control experiment pPrC3-1 was cotransfected with calf thymus DNA (Fig. 2*a*, lane 1). To exclude the possibility that calf thymus DNA might titrate factors that might be needed for activation of the $\alpha_1$(III) collagen promoter, the levels of expression of this promoter were compared after cotransfection with equal quantities of DNA from calf thymus, pSV2-gpt [a plasmid in which the early promoter of SV40 is fused to the bacterial guanine phosphoribosyltransferase gene (*gpt*)], or pRSV-gpt (a plasmid in which the LTR of the RSV genome is fused to the *gpt* gene). Both promoters contain enhancer sequences that exert a strong *cis*-acting stimulation on homologous and heterologous promoters in NIH 3T3 cells (19). Figure 2*b* shows that the level of CAT is the same in each of these three cotransfection experiments, implying that the enhancer present on these

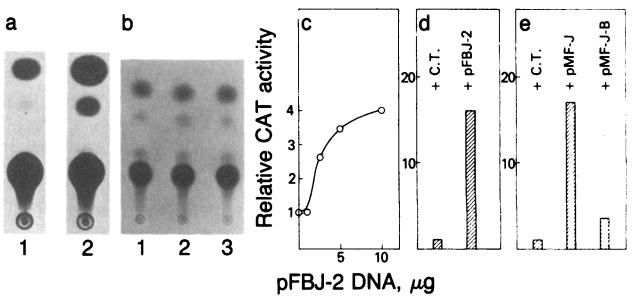


FIG. 2. *trans*-activation by v-*fos*. (*a*) Transfections were carried out with $5 \times 10^5$ NIH 3T3 cells per 100-cm$^2$ dish. Ten micrograms of pPrC3-1 DNA was cotransfected with either 10 μg of calf thymus DNA (lane 1) or 10 μg of pFBR-1 DNA (lane 2). After 48 hr, the levels of CAT were measured as described by Gorman *et al.* (15). An autoradiograph of the CAT reaction products after chromatography is shown. (*b*) Ten micrograms of pRC3-1 DNA was cotransfected with 10 μg of calf thymus DNA (lane 1), 10 μg of pSV2-gpt DNA (lane 2), or 10 μg of pRSV-gpt DNA (lane 3). (*c*) Three micrograms of pPrC3-1 was cotransfected with increasing amounts of pFBJ-2 DNA. The total amount of transfected DNA was kept constant at 20 μg by addition of calf thymus DNA. CAT was assayed as shown in *a* and *b*. (*d*) Ten micrograms of pPrC3-104 DNA (a derivative of pPrC3-1 without SV40 enhancer element) was cotransfected with 10 μg of calf thymus (C.T.) DNA or 10 μg of pFBJ-2 DNA. (*e*) Ten micrograms of pPrC3-1 was cotransfected with 10 μg of calf thymus (C.T.) DNA, 10 μg of MF-J DNA, or 10 μg of MF-J-B DNA plasmid carrying a frameshift mutation in the *fos* sequences. This experiment was done four times with very similar results.

plasmids has no effect on the expression of a gene present on the cotransfecting plasmid. A similar cotransfection was performed with pFBJ-2, a plasmid in which the *fos* sequences represent the only significant open reading that is common between the FBR and FBJ proviral sequences (18). Increasing concentrations of FBJ-2 DNA were used, whereas the concentration of pPrC3-1 DNA was constant and the total DNA concentration was kept equal by varying the concentrations of calf thymus DNA (Fig. 2*c*). In this particular concentration-dependent experiment the final stimulation was 4-fold. In three other experiments using the same plasmids, the stimulation was between 5- and 10-fold. These experiments are consistent with the notion that the *trans*-activation observed with both pFBR-1 and pFBJ-2 is encoded in the *fos* sequence. Fig. 2*d* shows that cotransfection of pFBJ-2 with a derivative of pPrC3-1 from which the SV40 enhancer sequences have been deleted results in a 15-fold stimulation of CAT activity. In the absence of the SV40 enhancer the unstimulated level of expression of the $\alpha_1$(III) collagen promoter is much lower than when the enhancer is present on the plasmid. The SV40 enhancer sequence is, therefore, not required to mediate the transactivation encoded in plasmid pFBJ-2.

To rule out that the *trans*-activating function is encoded by a non-*fos* segment common to the DNAs of pFBR-1 and pFBJ-2, plasmid pMF-J was constructed. In this plasmid a segment of pFBJ-2 DNA containing 81 bp preceding the AUG of v-*fos*, the v-*fos* coding segment, and 304 bp 3′ to the v-*fos* coding sequence was fused to the promoter segment of the mouse metallothionein I gene (19) (see Fig. 1). In addition, a derivative of pMF-J was made in which a frameshift mutation was introduced in the v-*fos* coding sequence. This was done by cleavage of pMF-J with *Bgl* II, which cleaves at a unique site in the *fos* coding sequence, followed by filling the staggered ends with the Klenow fragment of DNA polymerase and circularization of the plasmid by blunt-end ligation. In this plasmid, designated pMF-J-B, the first 60 amino acids of the fos polypeptide are intact but the subsequent sequences of fos are placed in a different translational frame as a result of a 4-bp insertion. This was confirmed by DNA sequence analysis. Fig. 2*e* shows the results of cotransfection experiments comparing the effects of calf thymus DNA, pMF-J DNA, and pMF-J-B DNA on the activity of the cotransfected $\alpha_1$(III) collagen promoter. In this experiment the levels of CAT are about 4 times higher when the collagen promoter plasmid is cotransfected with the wild-type *fos* DNA than with the mutated *fos* DNA. Although the mutation inhibits the *trans*-activation activity, it did not suppress it completely to the level observed in cotransfection of pPrC3-1 DNA and calf thymus DNA. This experiment was done four times, with very similar results each time. The mutation appears to exhibit some leakiness, which could be due either to the presence of frameshift suppressors in the cells or to reinitiation of translation in the fos RNA or, alternatively, to the fact that the transcriptional *trans*-activation activity is located in the amino-terminal portion of the v-fos protein.

Fig. 3 illustrates the results of transfection experiments in which the *trans*-activation effect of *fos* was tested on other promoters. In these experiments plasmid pFBJ-2 was cotransfected with plasmids in which the CAT gene is fused to the chicken β-actin promoter (pAZ1037) (21), the early promoter of SV40 (pSV2CAT), or the LTR promoter of RSV (pRSVCAT). Cotransfection with v-*fos* stimulates the activity of the RSV promoter (3.3 times) but not the activity of the actin or the early SV40 promoter. We conclude that not all promoters are equally *trans*-activated by v-*fos*. We also asked whether the stimulation of the RSV LTR promoter by v-*fos* was due to an increase in correctly initiated RNA. Fig. 3*d* shows the results of a primer extension experiment in which a primer was used that is complementary to a segment

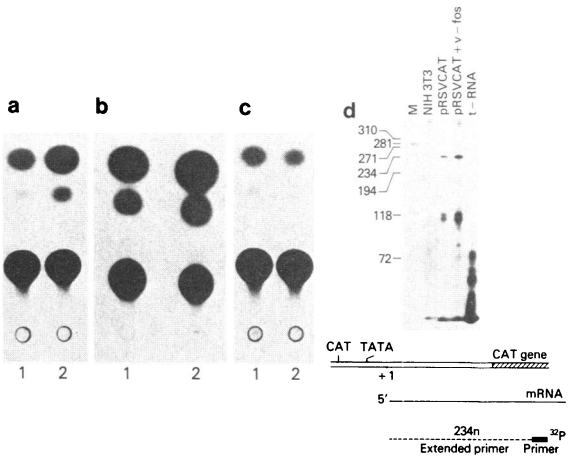


FIG. 3. *trans*-activation of other eukaryotic promoters by v-*fos*. All plasmids that were tested contain a different promoter segment linked to the CAT gene. (*a–c*) Ten micrograms of recombinant DNA plasmids carrying the different promoter sequences was cotransfected into $5 \times 10^5$ NIH 3T3 cells with 10 μg of calf thymus DNA (lane 1) or with 10 μg of pFBJ-2 DNA (lane 2). CAT reaction products are shown. (*a*) pRSVCAT-1. This plasmid contains the RSV LTR fused to the CAT gene. (*b*) pAZ1037. This plasmid contains a fragment of the chicken β-actin gene, which spans 350 bp upstream of the transcription start site and 1 kb downstream of this site (21). (*c*) pSV2CAT. This plasmid contains the early SV40 promoter segment, including the SV40 enhancer sequences, fused to the CAT gene (15). The ratios of CAT activity found in v-*fos*-transfected cells over that found in control cells are 3.4 for pRSVCAT, 1.3 for pAZ1037, and 0.7 for pSV2CAT. (*d*) Levels of CAT RNA in untransfected NIH 3T3 cells, cells cotransfected with 6 μg of pRSVCAT-1 and 20 μg of pBR322 DNA (pRSVCAT lane), or cells cotransfected with 6 μg of pRSVCAT-1 and 20 μg of pMF-J DNA (pRSVCAT + v-*fos* lane). Transfections were carried out in 150-cm$^2$ dishes. Forty-eight hours after transfection, total RNAs were isolated (16) and used for primer extensions, utilizing a 24-mer synthetic probe, labeled at its 5′ end, that is complementary to a segment proximal to the 5′ end of CAT mRNA (see map below the gel autoradiograph). The expected size for a cDNA extending to the 5′ end of the mRNA correctly initiated at the LTR promoter is 234 nucleotides (n). Lane M, size markers (in nucleotides); lane t-RNA, products of a reverse transcription reaction using excess yeast tRNA.

of CAT RNA. If CAT RNA starts at the correct place in the RSV promoter, the extended primer should be 234 nucleotides long. There is an intense doublet at 118 nucleotides that is probably due to a strong stop caused by a stretch of 17 G·C base pairs that are present in the DNA at this location as a result of the cloning procedure. In cells cotransfected with v-*fos* there is a clear increase in the intensity of the 234-nucleotide band, indicating that v-*fos* stimulates specific and correct initiation at the RSV promoter.

We have also examined the effects of v-*fos* in cotransfection experiments using a series of deletion-substitution mutations in the RSV promoter. The mutations are located between the *Sph* I site at −138 and the cap site at +1 (see Fig. 1). These mutations will be described in greater detail elsewhere (R.F., unpublished results). Several mutants, including C3 and C723, are stimulated by v-*fos* in a similar fashion as the wild-type RSV promoter (Fig. 4). Mutant C3 contains a deletion between −73 and −52 with an 8-bp substitution, whereas mutant C723 contains a deletion between −57 and −23 with an 8-bp substitution. The promoter activities of C3 and C723 were assayed after DNA transfection of plasmids in which these mutants were fused to the CAT gene. In other experiments it was shown that C3 has a promoter activity that is about 20% of that of the wild-type promoter, and the corresponding figure for C723 is about 10%. Two other mutations were tested and found to respond to *fos* in a similar fashion (not shown). These mutants are

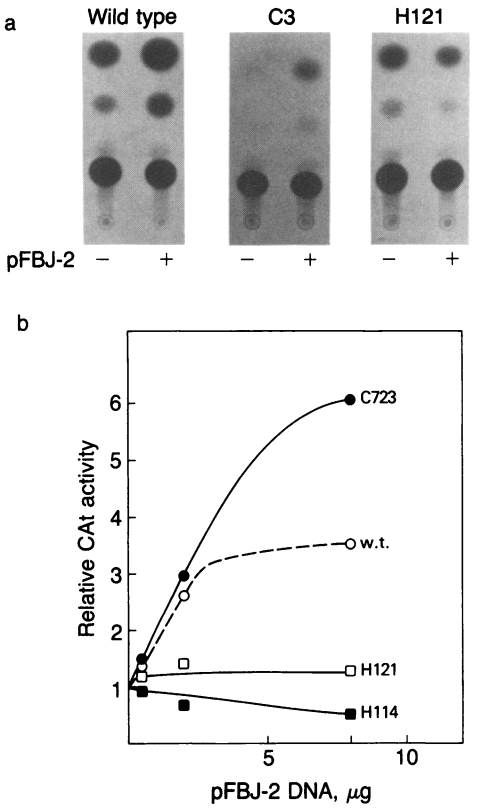


FIG. 4. Failure of some LTR promoter mutants to respond to the v-*fos*-mediated *trans*-activation. Transfections were carried out as for Fig. 2. (*a*) Ten micrograms of wild-type pRSVCAT-1 DNA (left), C3 DNA (center), or H121 DNA (right) was cotransfected with 10 μg of calf thymus DNA (− lanes) or 10 μg of pFBJ-2 DNA (+ lanes), and the results of the CAT assays of the transfectants are shown. (*b*) Increasing concentrations of pFBJ-2 DNA (0, 0.5, 2, and 8 μg) were cotransfected with 1 μg of wild-type pRSVCAT-1 DNA, C723 DNA, H121 DNA, or H114 DNA. Total amount of transfected DNA was maintained constant (9 μg) by addition of pBR322 DNA.

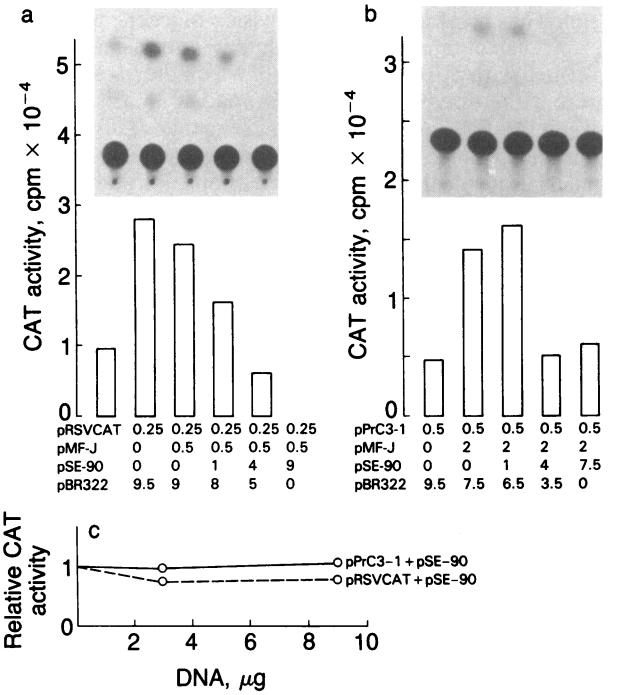


FIG. 5. Inhibition of the v-*fos*-induced promoter stimulation by cotransfection with increasing concentrations of a fragment of the RSV LTR promoter. (*a*) CAT activity in cells cotransfected with pRSVCAT-1 DNA, pMF-J DNA, and increasing concentrations of pSE-90 DNA. The amounts of each DNA are indicated in micrograms. The total amount of DNA was kept equal in each cotransfection with various concentrations of pBR322 DNA. The values in the diagram correspond to the cpm of acetylated chloramphenicol shown on the autoradiograph above. (*b*) CAT activity in cells cotransfected with pPrC3-1, pMF-J, and increasing concentrations of pSE-90 DNA. The amounts of each DNA are indicated in micrograms. (*c*) Relative levels of CAT activity in NIH 3T3 cells cotransfected with pPrC3-1 DNA (1 μg) + pSE-90 DNA (0, 3, and 9 μg) and with pRSVCAT-1 DNA (0.25 μg) + pSE-90 DNA (0, 3, and 9 μg). The total transfected DNA was kept constant at 10 μg and 9.25 μg, respectively, by addition of pBR322 DNA. Transfections were performed as for Fig. 2.

deletion substitutions that remove bases between −57 and −32 and between −57 and +8, substituting the same sequence as in C3 and C723. However, two mutants, H114 and H121, are not responsive to v-*fos*, suggesting that a specific site in the LTR promoter may be the target of *trans*-activation by v-*fos*. Both mutants are short deletion-substitutions that map in a small area of the promoter between −98 and −113. In other experiments, it was shown that the promoter strength of these mutants is 40% of wild-type for H114 and 65% for H121 (R.F., unpublished results).

To further characterize the possible interactions between this area of the RSV promoter and either v-*fos* itself or proteins induced by v-*fos*, an 87-bp fragment located between the *Sph* I site at −138 and the *Eco*RI site at −51 was subcloned in pBR322. This plasmid, pSE-90, was used in competition experiments to determine whether the stimulation by v-*fos* could be inhibited by adding increasing concentrations of this plasmid in cotransfection experiments. As shown in Fig. 5*c*, the 87-bp fragment itself does not inhibit the activity of either the RSV or the $\alpha_1$(III) collagen promoter. However, when the RSV promoter–CAT chimeric gene is cotransfected with the v-*fos*-containing plasmid pMF-J and increasing concentrations of pSE-90, the plasmid containing the 87-bp RSV sequence inhibits the stimulation that is produced by v-*fos*. In this experiment the total DNA concentration was kept constant by varying the concentrations of pBR322 DNA (Fig. 5*a*), and the 87-bp RSV fragment does not inhibit v-*fos* RNA synthesis (not shown). This experiment suggests that an activator of transcription is titrated by increasing concentrations of the 87-bp DNA fragment. The inhibition occurs only in these experiments in which the v-*fos* DNA plasmid is used. The inhibition by pSE-90 also occurs when the test promoter is the $\alpha_1$(III) collagen promoter (Fig. 5*b*). Taken together with the absence of response to v-*fos* of two closely linked mutations in the RSV promoter, the results of the competition experiment illustrated in Fig. 5 are consistent with the view that the 87-bp fragment of the RSV promoter is the target site either for *fos* itself or for a factor that is induced by v-*fos*.

## DISCUSSION

Our data show that the product of the v-*fos* oncogene has a transcriptional *trans*-activation activity. This activity was assayed by cotransfection of NIH 3T3 cells in transient expression experiments. The *trans*-activation was observed with three different v-*fos*-containing plasmids. The most convincing evidence for assigning a *trans*-activation function to v-*fos* comes from the observation that a frameshift mutation in the v-*fos* gene inhibits the *trans*-activation function. The two test promoters that exhibit a stimulation are the promoters for the $\alpha_1$(III) collagen gene and the LTR promoter of RSV. Since the test promoters that we used in our cotransfection experiments were fused to the CAT gene, we have measured increases in CAT activity, assuming that the levels of CAT activity reflect the activities of the linked

promoters (21, 24). With the RSV LTR promoter–CAT chimeric gene, we have in addition shown that there was a *fos*-mediated increase in correctly initiated RNA. The *trans*-activation appears, therefore, to stimulate specific transcription and not transcription from additional nonphysiological start sites. The finding that two mutants in the RSV promoter that map close to each other are not responsive to v-*fos*, whereas other mutants in this promoter respond to *fos*, like the wild-type promoter, strongly suggests that either v-*fos* itself or a protein induced by v-*fos* recognizes the sequence where these mutations map. The results of competition experiments, presented in Fig. 5, are consistent with this view.

The $\alpha_1$(III) collagen promoter–CAT chimeric gene lacking the SV40 enhancer in the plasmid was, in general, more strongly stimulated by v-*fos* than the RSV LTR–CAT gene and the $\alpha_1$(III) collagen promoter–CAT gene containing the SV40 enhancer. CAT gene expression with the latter plasmids has a much higher basal level than with the former plasmid. Hence, *trans*-activation by v-*fos* may be more easily detectable with promoters that do not have a high level of constitutive expression in NIH 3T3 cells.

The sequence around the RSV promoter mutations was compared with the sequence in the $\alpha_1$(III) collagen promoter between −350 and +1. No obvious similarities were found except for a short sequence 5′-ACAGACA-3′, which is present between −89 and −83 in the RSV promoter and is also found either intact (once) or with one or two base changes (twice) in the $\alpha_1$(III) promoter sequence. It is possible that the two mutations that do not respond to *fos* and that map at a short distance 5′ to this sequence cover only a portion of the recognition site and that the above heptanucleotide is another part of the interaction site. A deletion analysis of the $\alpha_1$(III) collagen promoter should help determine which sequences are needed in this promoter to observe the effects of v-*fos*.

A similar *trans*-activation function has been demonstrated for the protein products of other "oncogenes" such as the E1A protein of adenovirus (25–28), the large tumor proteins of the SV40 and polyoma viruses (29, 30), and the product of the c-*myc* gene (31). The genomes of human T lymphotropic viruses HTLV-I, HTLV-II, and HTLV-III and the genome of the bovine leukemia virus encode a gene product that can *trans*-activate the LTR of the homologous viruses (32–35). All these oncogenic products have a common nuclear localization. The genome of RSV also codes for a *trans*-activation function, but this function is not encoded by the *src* gene (36).

Our experiments do not allow us to determine whether the v-*fos* product itself stimulates the $\alpha_1$(III) collagen promoter and the RSV promoter or whether v-*fos* induces an activator of these promoters. DNA binding experiments using purified preparation of *fos* should help elucidate this point.

In NIH 3T3 cells that are transformed by the plasmid containing the FBR proviral sequences, there is a considerable increase in both the steady-state levels and synthesis of $\alpha_1$(III) collagen RNA (C.S., unpublished). This increase could be caused by the same *trans*-activating function that is found associated with v-*fos* in transient expression experiments. This hypothesis is supported by the finding that transfection of NIH 3T3 cells with v-*fos* results within 48 hr in an increase in the levels of endogenous type III collagen RNA. We suspect that the type III collagen gene is one among a series of cellular genes whose expression is influenced by v-*fos*. Type III collagen is one of the first collagens to be made in mouse embryos, and its appearance can first be detected at a pre-implantation stage well before the appearance of type I collagen (37).

We are grateful to Tom Curran for providing us with plasmids pFBJ-2 and pFBR-1. We thank Joyce Sharrar and Jennie Evans for preparation of the manuscript.

1. Miller, A. D., Curran, T. & Verma, I. M. (1984) *Cell* **36,** 51–60.
2. Finkel, M. P., Biskis, B. O. & Kinkins, P. B. (1966) *Science* **151,** 698–701.
3. Finkel, M. P., Reilly, C. A., Jr., & Biskis, B. O. (1975) *Front. Radiat. Ther. Oncol.* **10,** 28–39.
4. Curran, T. & Teich, N. M. (1982) *J. Virol.* **42,** 114–122.
5. Jenuwein, T., Müller, D., Curran, T. & Müller, R. (1985) *Cell* **41,** 629–637.
6. Curran, T., Miller, A. D., Zokas, L. & Verma, I. M. (1984) *Cell* **36,** 259–268.
7. Mitchell, R. L., Zokas, L., Schreiber, R. D. & Verma, I. M. (1985) *Cell* **40,** 209–217.
8. Müller, R., Müller, D. & Guilbert, L. (1984) *EMBO J.* **3,** 1887–1890.
9. Curran, T. & Morgan, J. I. (1985) *Science* **229,** 1265–1268.
10. Müller, R. & Wagner, E. F. (1984) *Nature (London)* **311,** 438–442.
11. Greenberg, M. E. & Ziff, E. B. (1984) *Nature (London)* **311,** 433–438.
12. Kruijer, W., Cooper, J. A., Hunter, T. & Verma, I. M. (1984) *Nature (London)* **312,** 711–716.
13. Cochran, B. H., Zullo, J., Verma, I. M. & Stiles, C. D. (1984) *Science* **226,** 1080–1082.
14. Müller, R., Bravo, R., Burckhardt, J. & Curran, T. (1984) *Nature (London)* **312,** 716–720.
15. Gorman, C. M., Moffat, L. & Howard, B. H. (1982) *Mol. Cell. Biol.* **2,** 1044–1051.
16. Chirgwin, J. M., Przybyla, A. E., MacDonald, R. J. & Rutter, W. J. (1979) *Biochemistry* **18,** 5294–5299.
17. Vogeli, G., Ohkubo, H., Sobel, M. E., Yamada, Y., Pastan, I. & de Crombrugghe, B. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 5334–5338.
18. Van Beveren, C., Enami, S., Curran, T. & Verma, I. M. (1984) *Virology* **135,** 229–243.
19. Curran, T., Peters, G., Van Beveren, C., Teich, N. & Verma, I. M. (1982) *J. Virol.* **44,** 674–682.
20. Yamamoto, T., Tyagi, S., Fagan, J. B., Jay, G., de Crombrugghe B. & Pastan, I. (1980) *J. Virol.* **35,** 436–443.
21. Schmidt, A., Setoyama, C. & de Crombrugghe, B. (1985) *Nature (London)* **314,** 286–289.
22. Liau, G., Yamada, Y. & de Crombrugghe, B. (1985) *J. Biol. Chem.* **260,** 531–536.
23. Law, M.-F., Byrne, J. C. & Howley, P. M. (1983) *Mol. Cell. Biol.* **3,** 2110–2115.
24. Gorman, C. M., Merlino, G. T., Willingham, M. C., Pastan, I. & Howard, B. H. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 6777–6781.
25. Treisman, R., Green, M. R. & Maniatis, T. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 7428–7432.
26. Green, M. R., Treisman, R. & Maniatis, T. (1983) *Cell* **35,** 137–148.
27. Gaynor, R. B., Hillman, D. & Berk, A. J. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 1193–1197.
28. Svensson, C. & Akusjarvi, G. (1984) *EMBO J.* **3,** 789–794.
29. Keller, J. M. & Alwine, J. C. (1984) *Cell* **36,** 381–389.
30. Brady, J., Bolen, J. B., Radonovich, M., Salzman, N. & Khoury, G. (1984) *Proc. Natl. Acad. Sci. USA* **81,** 2040–2044.
31. Kingston, R. E., Baldwin, A. S. & Sharp, P. A. (1984) *Nature (London)* **312,** 280–282.
32. Sodroski, J. G., Rosen, C. A. & Haseltine, W. A. (1984) *Science* **225,** 381–385.
33. Sodroski, J., Rosen, C., Wong-Staal, F., Salahuddin, S. Z., Popovic, M., Arya, S., Gallo, R. C. & Haseltine, W. A. (1985) *Science* **227,** 171–173.
34. Rosen, C. A., Sodroski, J. G., Kettman, R., Burny, A. & Haseltine, W. A. (1985) *Science* **227,** 320–322.
35. Derse, D., Carodonna, S. J. & Casey, J. W. (1985) *Science* **227,** 317–320.
36. Broome, S. & Gilbert, W. (1985) *Cell* **40,** 537–546.
37. Sherman, M. I., Gray, R., Gay, S. & Miller, E. J. (1980) *Dev. Biol.* **74,** 470–478.