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Gene Regulation by Tyrosine Kinases: *src* Protein Activates Various Promoters, Including c-*fos*

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A promoter of the nuclear proto-oncogene *fos* was activated by cotransfection with the viral *src* gene. Ability to transactivate the c-*fos* promoter was dependent on tyrosine kinase activity, because (i) *src* mutants which have reduced tyrosine kinase activity due to mutation of Tyr-416 to Phe showed lower promoter activation, (ii) pp60$^{c\text{-}src}$ mutants which have increased tyrosine kinase activity due to mutation of Tyr-527 to Phe also augmented c-*fos* promoter induction, and (iii) mutation in the ATP-binding site of pp60$^{v\text{-}src}$ strongly suppressed c-*fos* promoter activation. Tyrosine kinase activity alone, however, was not sufficient for promoter activation, because a pp60$^{v\text{-}src}$ mutant which lacked its myristylation site and consequently membrane association showed no increased c-*fos* promoter activation. Both the tyrosine kinase- and membrane-association-defective mutants were also unable to induce transformation. Therefore, phosphorylation of membrane-associated substrates appears to be required for both gene expression and cellular transformation by the *src* protein. Two regions of the c-*fos* promoter located between positions −362 and −324 and positions −323 and −294 were responsive to *src* stimulation. We believe that protein tyrosine phosphorylation represents an important step of signal transduction from the membrane to the nucleus.

A wide variety of agents which promote growth and differentiation induce the transcription of a number of nuclear proto-oncogenes (11, 21, 34, 39, 45, 47, 56, 57). The signal for induction of gene expression is transduced through a cascade of intracellular events initiated by the external stimulus. The precise mechanism of signal transduction remains largely conjectural but involves the participation of at least two major signal pathways. One employs the second-messenger cyclic AMP, while the other employs a combination of second messengers that includes Ca^{2+} ions and two substances, inositol triphosphate and diacylglycerol (41). Transduction may also proceed via protein-tyrosine kinases, several of which have been proposed as intermediaries of signal transmission from the membrane to the nucleus (26; J. M. Cooper, *in* B. Kemp and P. F. Alewood, ed., *Peptides and Protein Phosphorylation*, in press). pp60$^{c\text{-}src}$ (the product of the *src* proto-oncogene) is one of the best-studied protein-tyrosine kinases and is located at the cytoplasmic face of the plasma membrane where it may interact with receptor proteins (26; Cooper, in press). For instance, phosphorylation of pp60$^{c\text{-}src}$ on the amino-terminal tyrosine is increased after treatment of fibroblasts with platelet-derived growth factor (46).

A number of studies have shown that pp60$^{v\text{-}src}$, the activated viral form of the *src* protein, can increase (22, 55) or decrease (15, 25) the amounts of mRNAs encoding a wide variety of cellular proteins. In addition, both pp60$^{v\text{-}src}$ and other activated mutants of pp60$^{c\text{-}src}$ have been shown to induce expression of a protein having homology to a platelet mitogenic protein (53). One possibility that could explain these broad effects on gene regulation is that *src* protein first affects induction of nuclear transcriptional regulatory proteins which subsequently modulate transcription of other proteins. Both proto-oncogenes *fos* and *jun*(AP-1) are induced in response to a wide variety of agents (34, 45, 47, 56). The product of proto-oncogene *jun* binds to a specific DNA sequence referred to as the TPA (12-*o*-tetradecanoylphorbol-13-acetate) responsive element (TRE) (4). *fos* protein, on the other hand, does not directly bind to TRE but binds to AP-1 (49). The *fos*-AP-1 complex binds to TRE over 300-fold more avidly than AP-1 alone (49). Furthermore, cells cotransfected with *fos* and *jun*(AP-1) cDNAs show over 10-fold more transcriptional transactivation through TRE than that observed with *jun* alone (10, 48). In addition to being a transcriptional activator, *fos* protein can also act as a negative regulator of transcription (50). We therefore decided to study whether the *src* protein can directly mediate the induction of the nuclear proto-oncogene *fos*. Since both the viral and c-*src* proteins display tyrosine kinase activity, is there a correlation between enzymatic activity and gene expression? Finally, we wanted to investigate whether the membrane association of pp60$^{v\text{-}src}$ required for cellular transformation is also obligatory for inducing gene expression. Our results suggest a strict correlation of tyrosine kinase activity and membrane association of *src* protein with gene expression.

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MATERIALS AND METHODS

Plasmids. Most of the plasmids, including the *src* expression plasmid and CAT constructs used here, have previously been characterized (13, 18, 27, 29, 33, 42, 43; D. Valerio, V. W. van Beusechem, M. P. W. Einerhand, P. M. Hoogerbrugge, H. van der Putten, P. W. Wamsley, T. M. Berkvens, I. M. Verma, R. E. Kellems, and D. W. van Bekkum, Exp. Hematol., in press). The structures of pMv*src*, pM5HHB5, pATV-8, and pLSDLSDWT are shown in Fig. 1A. Plasmids pc*src*416, pc*src*527, and pc416527 are derived from pM5HHB5 but contain a Tyr→Phe mutation(s) at residues 416, 527, and both sites, respectively (33). pATV-8 is a plasmid in which viral DNA from the Prague strain of Rous sarcoma virus subgroup A had been cloned into pBR322 (31). SD2 and SD3 are derivatives of pATV-8 and contain a single point mutation at residue 295 (Lys→Arg and Lys→His, respectively) (29). pLSDLSDWT is a plasmid in

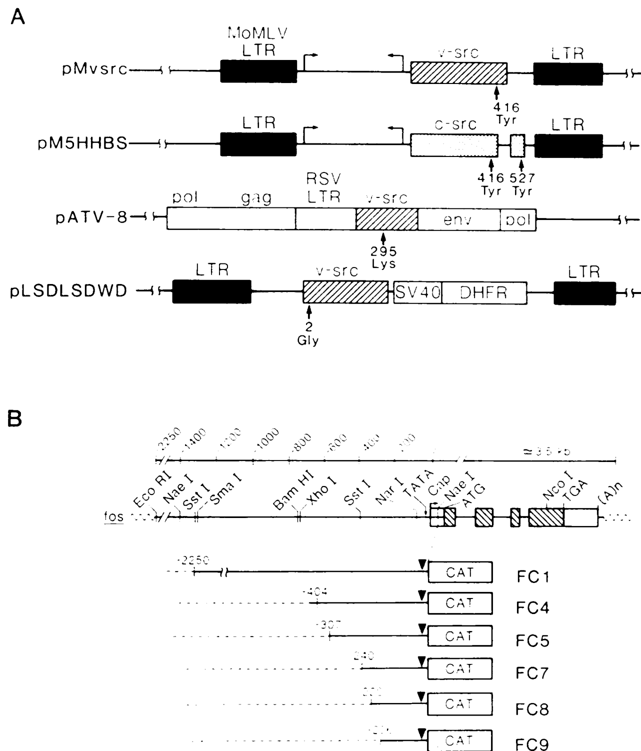


FIG. 1. Diagram of *src* expression vectors and *fos*-CAT constructs. (A) pMv*src*, pM5HHB5, pATV-8, and pLSDLSDWT. The closed box indicates the Moloney murine leukemia virus LTR. The splice donors and splice acceptor sites are indicated by bent arrows. Coding regions are shown by hatched (v-*src*) and stippled (c-*src*) boxes. The sites of mutations are indicated. In pLSDLSDWT, dihydroxyfolate reductase (DHFR) proteins are expressed by the SV40 early promoter. RSV, Rous sarcoma virus. (B) *fos*-CAT constructs (FC1 through FC9). The transcription initiation site (cap), the TATA box, the exons (hatched boxes), and several restriction enzyme sites are shown. kb, Kilobases.

which the v-*src* coding region (*Xho*I to *Xho*I) from pATV-8 was cloned into the Moloney murine leukemia virus expression vector pLSDL at a unique *Xho*I site. pLSDLSD10 is derived from pLSDLSDWT but contains a Gly→Ala mutation at residue 2. The *fos*-CAT constructs (FC1 through FC9) contain various c-*fos* promoter sequences from positions −2250 (FC1) through −206 (FC9) to position +42 (13). Similarly, the c-*myc*-CAT construct contains a c-*myc* promoter sequence between −2325 and +36 (18); the c-Ha-*ras*-CAT construct contains the c-Ha-*ras* sequence between positions −420 and +130 (27); pCHL4 (referred to as HTLV-I LTR) contains human T-cell lymphotropic virus type I (HTLV-I) long terminal repeat (LTR) sequence between positions −325 and +306 (42); HIV-CAT contains a human immunodeficiency virus (HIV) LTR sequence between positions −633 and +185 (43). ADA-CAT contains the 132-base-pair (bp) promoter of the adenosine deaminase gene, as well as all of its 5′ untranslated sequences, fused to the chloramphenicol acetyltransferase (CAT) gene (Valerio et al., in press). Plasmids pBSV, pCSV, pDSV, pFSV, pGSV, pPSV, and pQSV were constructed by the insertion of each of seven fragments from −362 to −220 of the c-*fos* promoter into plasmid pSVICCAT (see Fig. 3B). Each fragment was cloned in the sense orientation with respect to the simian virus 40 (SV40) early promoter. pSVICCAT is derived from pSV2CAT, but a 322-bp major enhancer region (between the *Acc*I and *Sph*I sites) in pSV2CAT was deleted (42).

Cell culture and DNA transfection. HeLa cells were cultured in Dulbecco modified Eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS). For transfection, HeLa cells were plated at 5×10^5 cells per 10-cm plate, and 24 h later, cells were transfected with 5 μg of CAT constructs and 5 to 15 μg of the *src* expression plasmid or control plasmids by calcium phosphate coprecipitation techniques (51). The cells were exposed to the precipitate for 24 h, washed with phosphate-buffered saline once, and further cultured in low (0.5% FCS–DMEM) or high (10% FCS–DMEM) serum for 16 h. CAT activities were determined as described previously (20) and quantified by cutting out spots containing the acetylated form of [^{14}C]chloramphenicol from the plate. Fold activation shows the ratio of CAT activity in the cells cotransfected with pMv*src* to that in cells cotransfected with pGEM-4 and is an average of a series of separate experiments.

RNA analysis. Cytoplasmic RNA was extracted from cells transfected with pMv*src*, pEVX, or pGEM-4 together with *fos*-CAT (FC4). RNase protection analysis was performed as described previously (58). Briefly, approximately 30 μg of RNA was hybridized to a specific ^{32}P-labeled cRNA probe at 45°C in 80% formamide–40 mM PIPES [piperazine-*N*,*N*′-bis(2-ethanesulfonic acid)] (pH 6.4)–400 mM NaCl–1 mM EDTA. Following 16 h of hybridization, samples were treated with RNase A (40 μg/ml) plus RNase T_1 (700 U/ml) at 32°C for 60 min. The samples were further incubated for 15 min at 37°C after addition of 1% sodium dodecyl sulfate and 50 μg of proteinase K. Following phenol-chloroform extraction and ethanol precipitation, samples were loaded on an 6% acrylamide–42% urea sequencing gel (a 340-nucleotide (nt) cRNA from *fos*-CAT was used as a probe).

RESULTS

Activation of c-*fos* promoter by pp60$^{v\text{-}src}$. To examine a possible role for pp60$^{v\text{-}src}$ in gene expression, we used a transient cotransfection assay with the c-*fos* promoter linked to the reporter gene, along with a plasmid capable of generating pp60$^{v\text{-}src}$. The plasmid, pMv*src*, contains a v-*src* gene cloned into the Moloney murine leukemia virus expression vector pEVX (Fig. 1A) (33). pMv*src* was transfected into HeLa cells along with plasmid FC4, which has c-*fos* promoter linked to the CAT gene (Fig. 1B) (13). CAT activity from the c-*fos* promoter was substantially increased by cotransfection with pMv*src* compared with the pGEM-4 control plasmid (Fig. 2A, lanes 1 and 2). Although serum augmented c-*fos* promoter activity (compare lanes 1 and 4), activation by pMv*src* was detected at either a low (0.5% FCS) or high (10% FCS) serum concentration, indicating that the effect of pMv*src* on the c-*fos* promoter is serum independent. To rule out the possibility that the Moloney murine leukemia virus LTR titrates out negative factors interacting with the c-*fos* promoter, the expression vector pEVX was transfected into HeLa cells with the *fos*-CAT plasmid. No stimulatory effect on the c-*fos* promoter was detected with pEVX (compare lanes 1, 3, 4, and 6). Therefore, the observed activation of the c-*fos* promoter by cotransfection with pMv*src* is due to pp60$^{v\text{-}src}$. The stimulation of the c-*fos* promoter was also detected in PC12 rat pheochromocytoma cells and K562 human erythroleukemia cells (data not shown).

Activation of the c-*fos* promoter by pp60$^{v\text{-}src}$ was further confirmed by RNase protection analysis. In agreement with

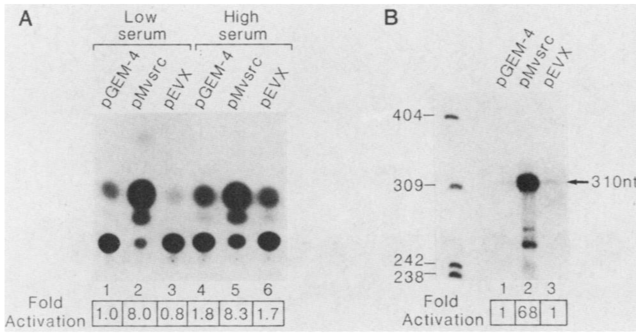


FIG. 2. Activation of the c-*fos* promoter by pp60$^{v\text{-}src}$. (A) CAT activity. HeLa cells were cotransfected with *fos*-CAT (FC4) plus pGEM-4, pMv*src*, or pEVX by the calcium phosphate coprecipitation technique as described in the text. After 24 h the cells were washed and cultured in a low (0.5% FCS) or high (10% FCS) concentration of serum. CAT activity was assayed from extracts prepared after further incubation for 16 h. (B) *fos*-CAT RNA transcripts. HeLa cells were cotransfected with *fos*-CAT (FC4) plus pGEM-4, pMv*src*, or pEVX. After 24 h the cells were washed and cultured in 0.5% FCS-DMEM for 16 h, and cytoplasmic RNA was extracted and hybridized with a 340-nt ^{32}P-labeled cRNA probe from *fos*-CAT. The expected size after digestion with RNase is 310 nt.

the results of CAT assays with cell extracts, increased amounts of a 310-nt correctly initiated *fos*-CAT transcript was detected following cotransfection with pMv*src* (Fig. 2B, lane 2). In contrast, no increased *fos*-CAT transcripts could be observed with pEVX (lane 3). It should be pointed out that the RNase protection analysis shown in Fig. 2B measures steady-state RNA levels and not rates of transcription. Given the data on the de novo transcription of the c-*fos* gene following induction with serum (21) or phorbol ester (TPA; 39), it is, however, likely that v-*src*-mediated induction also occurs at the transcriptional level.

Sequences in c-*fos* upstream region required for activation by pp60$^{v\text{-}src}$. To identify the c-*fos* promoter upstream sequences mediating activation by pp60$^{v\text{-}src}$, a series of plasmids containing c-*fos* promoter 5′ deletion mutations linked to the CAT gene were used (Fig. 3A). The regions contained in these plasmids are shown in Fig. 1B. Increased CAT activities were observed when plasmids FC1, FC4, FC5, and FC7 were cotransfected with the v-*src* gene, but little or no activation was observed with constructs FC8 and FC9 (Fig. 3A). Thus, the sequences responsive to induction by pp60$^{v\text{-}src}$ are located between −404 and −220 (Fig. 1B and 3A). It is worth noting that the basal level (uninduced) is higher in FC4 than in FC1 (Fig. 3A). This has consistently been observed and is likely due to some negative sequence elements between −2250 and −404 (unpublished results). In general, plasmids containing a dyad symmetry element (DSE) show higher basal levels, presumably due to residual serum stimulation.

To further define this element, we cloned the 143-bp fragment between −362 and −220 of the c-*fos* promoter (B fragment) into a pSVICCAT vector to generate plasmid pBSV (Fig. 3B). pSVICCAT was derived from pSV2CAT, in which the CAT gene is under the control of the SV40 early promoter, by deleting a 322-bp major enhancer element (42). pp60$^{v\text{-}src}$ had little effect on CAT activity (twofold activation) with pSVICCAT (Fig. 3B). However, the CAT activity from the plasmid containing the B fragment was substantially increased (63-fold) by pp60$^{v\text{-}src}$. Activation by pp60$^{v\text{-}src}$ was also detected when the B fragment was inserted in

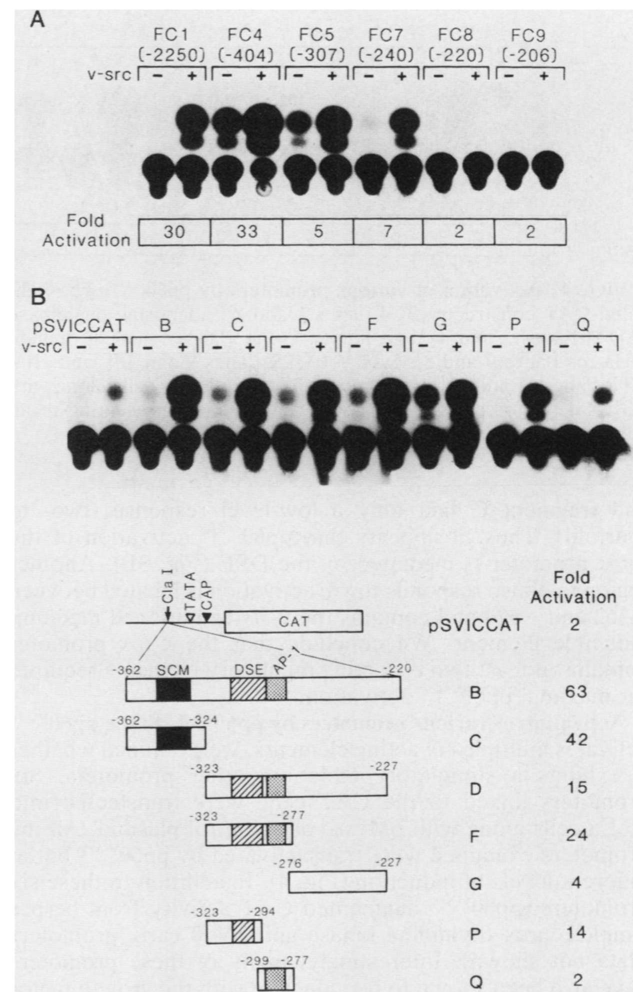


FIG. 3. Identification of the c-*fos* promoter region required for pp60$^{v\text{-}src}$ activation. (A) HeLa cells were cotransfected with FC1 through FC9 plus pGEM-4 (−) or pMv*src* (+). The structures of FC1 through FC9 are shown in Fig. 1. The fold activation and the 5′ endpoints of the deletions are indicated. The fold activation represents an average of three independent experiments. (B) Plasmid pSVICCAT, pBSV, pCSV, pDSV, pFSV, pGSV, pPSV, or pQSV was cotransfected in HeLa cells along with pGEM-4 (−) or pMv*src* (+). The regions of the *fos* upstream promoter region which are included and the fold activation are indicated. DSE, Dyad symmetry element; SCM, v-*sis*-conditioned medium-responsive element; AP-1, AP-1-like site.

pSVICCAT in the opposite orientation (data not shown). Therefore, the c-*fos* upstream element between −362 and −220 contains a *cis*-acting regulatory element required for pp60$^{v\text{-}src}$ activation. Since the 143-bp fragment contains a DSE (56), tax$_1$-responsive element (17), v-*sis*-conditioned medium-responsive element (24), and AP-1-like element (44), we cloned subfragments into the pSVICCAT vector such that these elements were individually separated (Fig. 3B). Strong activation was observed with C, D, and F subfragments (Fig. 3B). The 97-bp D fragment, which contains the DSE and AP-1-like sites, was further subdivided, and its fragments were molecularly cloned separately into pSVICCAT. The P fragment (−323 to −294), which contains only the DSE, was activated by pp60$^{v\text{-}src}$ (Fig. 3B). On the other hand, fragment Q, which contains the AP-1-like site,

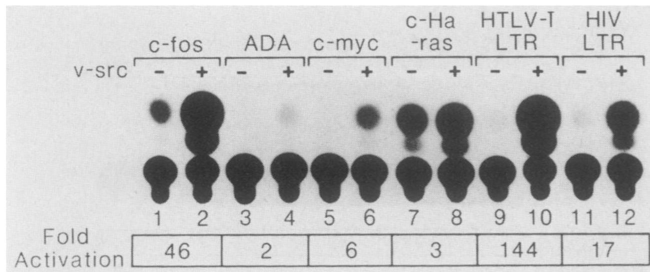


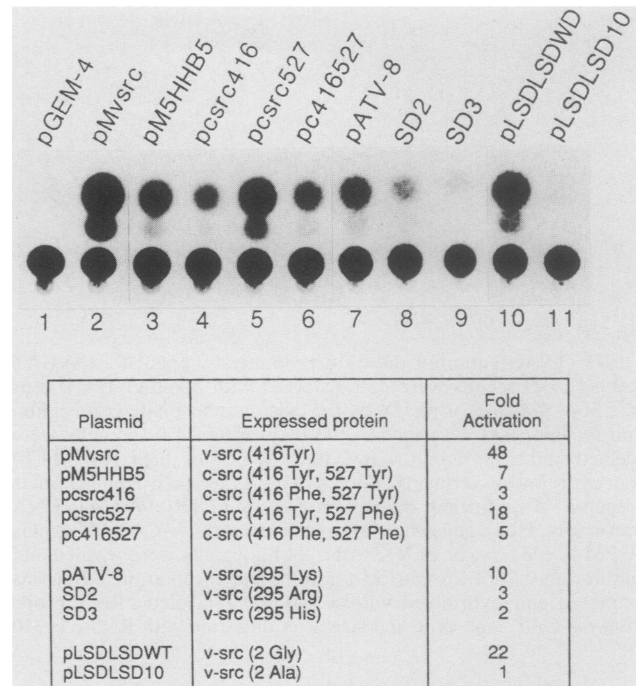
FIG. 4. Activation of various promoters by pp60$^{v\text{-}src}$. The indicated CAT constructs (FC4 [lanes 1 and 2], adenosine deaminase [ADA] [lanes 3 and 4], and human c-*myc* [lanes 5 and 6], human c-Ha-*ras* [lanes 7 and 8], HTLV-I LTR [lanes 9 and 10], and HIV LTR [lanes 11 and 12]) were cotransfected in HeLa cells along with pGEM-4 (−) or pMv*src* (+). Fold activations representing mean values from three independent experiments are indicated.

and fragment G had only a low-level response (two- to fourfold). Thus, it appears that pp60$^{v\text{-}src}$ activation of the c-*fos* promoter is mediated by the DSE (Fig. 3B). Another region that also responds to *src* activation is located between −362 and −324 and contains the v-*sis*-conditioned medium-inducible element. We conclude that the c-*fos* promoter contains at least two *cis*-acting regulatory elements required for maximal pp60$^{v\text{-}src}$ activation.

Activation of various promoters by pp60$^{v\text{-}src}$. Since pp60$^{v\text{-}src}$ activates multiple *cis*-acting elements, we examined whether it exhibits a stimulatory effect on other promoters. Six promoters linked to the CAT gene were transfected into HeLa cells along with pMv*src* or a control plasmid. All the promoters examined were transactivated by pp60$^{v\text{-}src}$ but at different levels of induction (Fig. 4). In addition to these six promoters, pp60$^{v\text{-}src}$ augmented CAT activity from herpes simplex virus thymidine kinase and SV40 early promoters (data not shown). Interestingly, many of these promoters have also been shown to be inducible with the growth factor TPA or cyclic AMP (16, 21, 32, 40). On the other hand, promoters for the adenosine deaminase gene or c-Ha-*ras*, which appear to represent housekeeping genes that are not stimulated by growth factors or agonists of the adenylate cyclase pathway, showed little response to pp60$^{v\text{-}src}$ (Fig. 4). It thus appears that gene expression in response to a variety of agents may involve common intermediates.

Requirement for tyrosine kinase activity. Both the viral and cellular *src* proteins manifest intrinsic tyrosine kinase activity (26). To examine a role for tyrosine phosphorylation in gene activation, several v- or c-*src* mutants were transfected into HeLa cells along with a *fos*-CAT (FC4) plasmid. The mutants used here have been previously described (29, 33) and are classified into two groups: (i) mutants with mutations at residue 527 (Tyr→Phe) and/or residue 416 (Tyr→Phe) which activate and repress *src* tyrosine kinase activity, respectively; and (ii) mutants with mutations of the ATP-binding site at residue 295 (Lys→Arg and Lys→His) which abolish tyrosine kinase activity.

These mutants were transfected into HeLa cells along with the *fos*-CAT (FC4) plasmid (Fig. 5). The activity of the c-*fos* promoter was augmented by pp60$^{c\text{-}src}$ (lane 3), but the level of the induction was lower than that by pp60$^{v\text{-}src}$ (compare lanes 2 and 3). Mutations which decreased tyrosine kinase activity (Tyr-416→Phe) also decreased c-*fos* promoter activation (lane 4). On the other hand, mutation at the regulatory tyrosine 527 (pc*src*527), which increased tyrosine kinase activity, correspondingly increased the in-



Plasmid	Expressed protein	Fold Activation
pMvsrc	v-src (416Tyr)	48
pM5HHB5	c-src (416 Tyr, 527 Tyr)	7
pcsrc416	c-src (416 Phe, 527 Tyr)	3
pcsrc527	c-src (416 Tyr, 527 Phe)	18
pc416527	c-src (416 Phe, 527 Phe)	5
pATV-8	v-src (295 Lys)	10
SD2	v-src (295 Arg)	3
SD3	v-src (295 His)	2
pLSDLSDWT	v-src (2 Gly)	22
pLSDLSD10	v-src (2 Ala)	1

FIG. 5. Activation of c-*fos* promoter by *src* mutants. The indicated *src* mutants were transfected in HeLa cells along with FC4. The structures of pMv*src*, pM5HHB5, pATV-8, and pLSDLSDWT are shown in Fig. 1. The other *src* expression plasmids contain point mutations as indicated in the panel. The fold activation is an average from three experiments.

duction from the c-*fos* promoter (lane 5). A double mutant, pc416527, with lower tyrosine kinase activity than pc*src*527 showed concomitantly reduced c-*fos* promoter stimulation (lane 6). These results imply that tyrosine kinase activity of *src* proteins is obligatory for the observed c-*fos* promoter activation.

The role of tyrosine kinase activity in gene activation was further confirmed by mutation of the pp60$^{v\text{-}src}$ ATP-binding site. While plasmid pATV-8, encoding wild-type Rous sarcoma virus, increased c-*fos* promoter activity (Fig. 5, lane 7), mutation of Lys to Arg (SD2) or Lys to His (SD3) at residue 295, which abolished the tyrosine kinase activity, had little stimulatory effect on c-*fos* promoter activity (Fig. 5, lanes 8 and 9). The data for the tyrosine kinase activity- and ATP-binding site-deficient mutants strongly suggest that tyrosine phosphorylating activity of *src* protein can regulate c-*fos* gene induction.

Requirement for membrane association. Both the v- and c-*src* proteins are myristylated at glycine 2. This is required for their association with the cell membrane (23, 28). Furthermore, membrane association is essential for their transforming activity but not for all mitogenic activity (7, 28). To investigate the dependence of c-*fos* gene activation on membrane association, two homologous v-*src* expression plasmids which express wild type v-*src* (pLSDLSDWT) or mutant v-*src* containing a Gly-2→Ala substitution (pLSDLSD10) were cotransfected with FC4 into HeLa cells. Wild-type pp60$^{v\text{-}src}$ increased c-*fos* promoter activity (Fig. 5, lane 10), but the mutant which was not associated with the membrane failed to exhibit any stimulatory activity on the c-*fos* promoter (lane 11). Since this mutant has tyrosine kinase activity similar to that of the wild-type pp60$^{v\text{-}src}$ (28), we presume that tyrosine phosphorylation of membrane-

associated substrates is required for pp60^{v-src} stimulation of the c-*fos* promoter.

DISCUSSION

Tyrosine kinases induce gene expression. Activation of gene expression is a common consequence following stimulation of receptor tyrosine kinases by growth factors like epidermal growth factor, platelet-derived growth factor, or colony-stimulating factor 1 (26; Cooper, in press). Early events following the addition of growth factors to cells include autophosphorylation of the receptor, activation of its intrinsic tyrosine kinase activity, and degradation of phosphatidylinositol (PI) resulting in the production of diacylglycerol and inositol triphosphate (26, 41). While diacylglycerol leads to the activation of protein kinase C and subsequent phosphorylation of specific proteins, inositol triphosphate triggers the release of Ca^{2+} from intracellular pools (42). Oncogenic forms of receptor tyrosine kinase may bypass the requirement for ligand binding to activate gene expression. For instance, transformation of NIH 3T3 cells by the oncogene v-*fms*, the viral homolog of the colony-stimulating factor 1 receptor gene, does not require cognate colony-stimulating factor 1 (14). We have addressed the issue of whether tyrosine kinase activity is required for gene expression by exploiting the use of wild-type and mutant *src* genes. The mutants induced various degrees of cell transformation and fall into three categories: (i) quantitatively altered tyrosine kinase activity, (ii) total absence of tyrosine kinase activity due to an inability to bind ATP, and (iii) lack of membrane association due to loss of the myristylation site. As an indicator of gene expression, we have used the c-*fos* promoter linked to a heterologous gene. Results shown in Fig. 5 clearly demonstrate that all the mutants with reduced tyrosine kinase activity also showed reduced stimulation of the c-*fos* promoter. Thus, it appears that tyrosine kinase activity may be an important intermediate step for transduction of the signal for *fos* gene expression. However, the direct role of c-*fos* gene expression in cellular transformation remains conjectural.

The data obtained with the myristylation mutant suggest that the tyrosine kinase activity does not directly regulate the induction of the c-*fos* gene (Fig. 5, lanes 10 and 11). This mutant has tyrosine kinase activity similar to the wild type but is unable to induce the c-*fos* gene or cause transformation (28). It follows that targets of *src* tyrosine kinase exist in association with membranes. In this context it is worth noting that in pLSDLSD10 (Gly-2→Ala)-mutant-infected cells, decreased levels of tyrosine phosphorylation of some membrane proteins were detected (28, 35), and an 81- to 85-kilodalton putative PI kinase has been suggested to be a substrate of *src* kinase activity (12, 30). Transforming proteins *src*, *ros*, and polymavirus middle T antigen have been shown to be associated with increased PI kinase activity, suggesting that PI kinase may be a substrate involved in gene regulation (8, 36, 54). Activation of PI kinase leads to PI hydrolysis, initiating a chain of biochemical events leading to the formation of intermediates essential in the generation of second messenger. Increased PI turnover following mitogenic stimulation of cells with epidermal growth factor or platelet-derived growth factor lends strong support to this notion (52).

Promoter elements required for induction with tyrosine kinase. Extensive delineation of the c-*fos* promoter revealed that sequences responsive to *src* induction were localized in the region between −404 and −220 upstream of the 5′ cap site (Fig. 3). This region contains both the DSE previously identified to be serum responsive and sequences required for induction with v-*sis* condition medium (24, 56). Constructs containing these sequences separately responded to pp60^{v-src}. The DSE has also been shown to be required for induction with phorbol esters, which leads to the activation of the protein kinase C pathway (56). Since at least six other promoters which do not contain the DSE also respond to activation with *src* protein, we presume that other, as yet undefined, sequences may be involved in induction. It should be pointed out that induction of the HIV LTR with TPA requires transcription factor NF-kB while induction of *fos* with TPA requires serum response factor (5, 56). Thus, it is not unusual for the same inducer to use different cellular machinery to elicit gene expression.

Pleiotropic function of *src*. pp60^{c-src} has been implicated in playing pivotal roles in cellular proliferation and differentiation (26). In addition to fibroblasts, pp60^{v-src} can transform erythroid cells (3) and convert myeloid cells from factor dependent (chicken myelomonocytic growth factor) to factor independent for proliferation (1). Neuronal cells express a modified form of c-*src* protein with a high level of kinase activity (6). Alema et al. (2) showed that Rous sarcoma virus infection of the rat pheochromocytoma cell line PC12 induces differentiation to neuronlike cells, similar to that observed upon treatment of PC12 cells with nerve growth factor. Furthermore, pp60^{c-src} kinase activity is enhanced during fibroblast mitosis, suggesting that it may regulate passage through the cell cycle (9). How does *src* protein potentiate such divergent activities? Clearly it can modulate expression of different genes. One scenario is that regulatory nuclear oncoproteins like c-*fos* and c-*myc* induced by *src* protein influence the regulation of other genes. Recently, it has been shown that *fos* protein can negatively regulate its own transcription and that of at least the heat shock promoter (50). On the other hand, the *fos* gene product can transactivate the transcription of genes containing TRE, the AP-1-inducible element (10, 51). Furthermore, it appears that *fos* and AP-1 proteins cooperate to achieve maximal induction (10, 48). Thus, it is possible that *src*-induced *fos* protein may act as a positive regulator by inducing gene expression or as a negative regulator by suppressing gene expression.

In fact, the activation or suppression of several genes in pp60^{v-src}-transformed cells has been reported (15, 19, 22, 25, 55). We believe that tyrosine kinases typified by *src* protein play important roles during cell growth and proliferation by modulating gene expression through a cascade of complex networks involving signal transduction from the membrane to the nucleus.

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