v-src Induces Prostaglandin Synthase 2 Gene Expression by Activation of the c-J un N-terminal Kinase and the c-J un Transcription Factor*

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A consensus cyclic AMP response element (CRE) in the murine prostaglandin synthase-2 (PGS2) promoter is essential for pgs2 gene expression induced by pp60v-src, the v-src oncogene product. In this study, we investigate (i) the transcription factors active at the PGS2 “CRE site” in response to v-src activation and (ii) the signal transduction pathways by which pp60v-src activates these transcription factors. Transient transfection assays with pgs2 promoter/luciferase reporter chimeric genes suggest that c-j un mediates v-src-induced pgs2 gene expression. Antibody supershift experiments demonstrate that c-j un can participate in a complex with the pgs2 promoter CRE site. Moreover, in vitro immunocomplex assays demonstrate that pp60v-src expression strongly activates c-j un N-terminal kinase (JNK1) enzyme activity. Serines 63 and 73, the sites of c-j un phosphorylation by JNK, are essential for v-src-induced, pgs2 promoter-mediated luciferase expression. Cotransfection studies with plasmids expressing wild-type JNK, dominant-negative JNK, and dominant-negative MEKK-1 confirm that activation of the Ras/MEKK-1/JNK pathway is required for v-src-induced pgs2 gene expression. Overexpression of either wild-type ERK-1 or ERK-2 proteins also potentiate v-src-mediated luciferase expression driven by the pgs2 promoter, and expression of dominant-negative mutants of ERK-1, ERK-2, or Raf-1 attenuate this response. Thus, in response to v-src expression, a Ras/MEKK-1/JNK signal transduction pathway activating c-j un and a Ras/Raf/1/ERK pathway converge to mediate pgs2 gene expression via the CRE site in the pgs2 promoter.

The prostaglandins play key roles in a variety of biological processes, including cell division, blood pressure regulation, immune responses, ovulation, bone development, wound healing, and water balance. Altered prostaglandin production is associated with several pathophysiological states, including immune responses, ovulation, bone development, wound healing, and water balance. The prostaglandins also play key roles in various pathophysiological states, including immune responses, ovulation, bone development, wound healing, and water balance. The prostaglandins also play key roles in various pathophysiological states, including immune responses, ovulation, bone development, wound healing, and water balance.

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1. The abbreviations used are: PGS, prostaglandin synthase; ATF, activating transcription factor; J NK, c-j un N-terminal kinase; ERK, extracellular signal-regulated kinase; CRE, cyclic AMP response element; CREB, cyclic AMP response element binding protein; EMGS, electrophoretic mobility gel shift; MAP, mitogen-activated protein.
and determine the signal transduction pathway that mediates the activation of this factor.

**EXPERIMENTAL PROCEDURES**

Plasmids—We previously referred to PGS2 as TIS10 (4, 9). Our promoter constructs thus historically have the TIS10 designation (8, 9). The reporter constructs pTIS10ΔLUC and pTIS10ΔLUC, as well as the v-src expression vector pMV-src were described previously (8). The overlapping CRE and E-box sites of the PGS2 regulatory region are located at nucleotides –56 to –48. Thus, the pTIS10ΔLUC contains the overlapping CRE and E-box cis-acting sites of the pGS2 promoter, while the pTIS10ΔLUC construct has these sites deleted. The plasmid pGAL4TIS10ΔLUC was constructed by ligating five GAL4 DNA binding sites to the 5′-end of the pTIS10ΔLUC reporter. Expression vectors of pRSV-CREB, GAL4-CREB (a fusion protein containing the GAL4 DNA binding domain fused to the dominant-negative M1 CREB mutant) (10) were the gifts of Dr. Marc Montminy (Salk Institute). The expression vectors pSRαMSVtkNeo-c-Jun and pSRαMSVtkNeo-Fos (11) were the gifts of Dr. Charles Sawyer (UCLA). The expression vectors for GAL4DB, GAL4-c-jun, and GAL4-c-jun 63/73 (a fusion protein containing the GAL4 DNA binding domain fused to a dominant-negative M1 CREB) were the gifts of Dr. Melanie Cobb (University of Massachusetts). The expression vectors pCEP4Erk1, pCEP4Erk2, pCEP4Erk1K379, and GST-c-Jun(amino acids 1–79) (15) were the gifts of Dr. Roger Davis (ONYX, CA). pCDNA-Flag-JNK1 and pCDNA-DN-JNK1, expression vectors for Flag-tagged JNK1 and kinase-defective JNK1, respectively, were prepared from UP1A1 cells, a 3T3 cell line carrying a temperature sensitive v-src gene (8). The nuclear extract used for gel shift in this study was prepared by digestion with leucines; Ref. 12) were gifts from Dr. Andrew Kraft (University of California, San Diego) (15). pEYX-3Rat AT35A, an expression vector that encodes a dominant-negative Raf-1 (16), was the gift of Dr. Susan Macdonald (ONYX, CA). pCDNA-Flag-c-Jun and pCDNA-DN-c-Jun, expression vectors for Flag-tagged c-Jun and kinase-defective c-Jun, respectively, and GST-c-Jun (amino acids 1–79) (15) were the gifts of Dr. Roger Davis (University of Massachusetts). The expression vectors pCEP4ERK1, pCEP4ERK1K379, and pCEP4ERK1K525, encoding wild-type ERK1, wild-type ERK2, dominant-negative ERK1, and dominant-negative ERK2, respectively, were the gifts of Dr. Melanie Cobb (University of Texas, Southwestern).

**Cells and Cell Transfection—**NIH 3T3 cells were grown and transfected as described previously (8). 3 µg of various reporter constructs and 1.5 µg of v-src expression vector pMV-src or the empty expression vector pEYX per 60-mm dish were used for all experiments. The amounts of other constructs are specified in each experiment. The luciferase assay was performed as described previously (8). Triplicate dishes were used for all transfections, and all experiments were repeated at least twice.

**Immunocomplex JNK Assay—**The JNK immunocomplex kinase assay was performed as described (15). 6 µg of plasmid encoding Flag-tagged c-Jun was transfected into NIH 3T3 cells in 100-mm dishes, using the calcium phosphate method (8), along with either 4 µg of pMV-src expression vector or 0.5 µg of empty vector pEYX. The cells were kept in 0.5% newborn calf serum for 24 h after transfection, then lysed in lysis buffer (25 mM HEPES, pH 7.5, 10% glycerol, 1% Triton X-100, 137 mM NaCl, 2 mM EDTA, 25 mM sodium glycoporphosphate, 10 µg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride). Extracts were pre-absorbed with Sepharose-4B beads (Pharmacia Biotech Inc.) for 30 min before the M2 monoclonal antibody (Eastman Kodak Co.) against Flag was added. A small sample of each extract from each cell lysate was taken for protein assay to standardize for gel loading after the kinase reaction. M2 antibody was mixed with Sepharose beads for 1.5 h in a cold room, and extra antibody was washed away using lysis buffer. Then, the antibody-conjugated beads were added to the preabsorbed cell lysate for another 1.5 h. The binding mixtures were washed three times with lysis buffer and twice with kinase assay buffer (15). The kinase assay was carried out in a 30-µl reaction mixture containing 20 mM HEPES, 20 mM MgCl2, 25 mM sodium glycoporphosphate, 100 µM sodium orthovanadate, 2 mM dithiothreitol, 20 µM ATP, 2 µg of GST-c-jun, 10 µCi of [γ-32P]ATP at 30 °C for 30 min. The reactions were terminated by adding SDS loading buffer and incubating in boiling water for 5 min. The reaction mixtures were subjected to electrophoresis on SDS-polyacrylamide gel electrophoresis gels, which were then dried and exposed to x-ray film.

**Ectodomain Mobility Gel Shift (EMGS) Assays—**Nuclear extracts were prepared from UP1A1 cells, a 3T3 cell line carrying a temperature sensitive v-src gene (8). The nuclear extract used for gel shift in this experiment was prepared from NIH 3T3 cells grown at the permissive temperature, where v-src is active. The gel shift and supershift protocols were as described previously (8), with the exception that the running buffer used in this study is composed of 10 mM HEPES (pH 8.0), 10 mM Tris, 5 mM EDTA, instead of Tris-glycine buffer. The c-jun and CREB binding protein antibodies were purchased from Santa Cruz Biotech.

**RESULTS**

**Overexpression of ATF Proteins Blocks v-src-mediated Induction from the pgs2 promoter**—Deletion and mutation analysis of the pgs2 promoter identified the CRE site CTGCA located at nucleotide –56 of the murine pgs2 gene as essential for v-src-induced pgs2 expression (8). EMGS supershift experiments with anti-CREB antibody demonstrated that CRE binds to this PGS2 CRE site (8). CREB-M1, a dominant-negative CREB mutant that binds to CRE sequences but has an inactive transactivation domain (10), blocks v-src-mediated PGS2 activation (8). We proposed that CREB and/or other member of the ATF transcription factor family might mediate v-src induction from the CRE site of the pgs2 promoter (8).

We first tested whether increasing the levels of wild-type CREB in cells could enhance v-src-mediated induction from the pgs2 promoter of the pTIS10ΔLUC chimeric reporter gene. This construct contains nucleotides –80 to +3 of the pgs2 promoter (we previously referred to PGS2 as TIS10 (4, 9); our promoter constructs thus historically have the TIS10 designation (8, 9)). However, overexpression of CREB, like overexpression of the dominant-negative CREB-M1 mutant, inhibits v-src-induced luciferase expression from the PGS2 reporter (Fig. 1). Because we were concerned that high levels of CREB might “squelch” CREB-dependent expression from the PGS2 CRE site (8), we also used lower concentrations of wild-type CREB expression vector (data not shown). Enhancement of luciferase expression from the pgs2 promoter by CREB was never observed. We conclude that some transcription factor other than CREB is activated by pp60v-src expression and elevates pgs2 gene expression by transcriptional activation from the PGS2 CRE site.

We next tested whether overexpression of other ATF transcription factors might modulate v-src induction from the pgs2...
The binding of proteins in nuclear extract to the E-box sequence of the E-box oligonucleotide competitor, we were able to reduce the contribution of the E-box complexes. The E-box binding complexes and the CRE binding complexes indicated in the figure have been identified previously, both by competition with consensus E-box and CRE sequences, and by EMGS-antibody supershift experiments (8). The c-Jun-containing complex is identified on the basis of the c-Jun antibody-dependent supershift.

Promoter. Overexpression of both ATF-2 and ATF-3, like CREB or CREB-M1 overexpression, also inhibited, rather than augmented, v-src-induced expression from the PGS2 CRE site (data not shown). We conclude that the ATF family members CREB, ATF-2, and ATF-3 do not mediate v-src induction of the pgs2 gene. The data presented in this section demonstrating that occupation of the PGS2 CRE site by CREB, DN-CREB, ATF-2, and ATF-3 block v-src-mediated pgs2 gene expression suggest (i) that this site is necessary for v-src-mediated induction of the pgs2 gene and (ii) that some transcription factor(s) other than these molecules must mediate this induction.

C-Jun Binds to the CRE Site of the pgs2 Promoter—EMGS and antibody-supershift experiments previously demonstrated that other, unidentified nuclear protein(s) in addition to CREB can bind to the PGS2 CRE site (8). c-Jun, a member of the bZIP transcription factor superfamily, can form heterodimers with members of the ATF family and bind to CRE sequences (18). Moreover, c-Jun and Fos heterodimers can also bind to CRE sequences (19). We investigated whether c-Jun might play a role in v-src-mediated induction of the pgs2 gene. We used antibody to c-Jun in a supershift experiment to determine if c-Jun is present in a complex that binds to the PGS2 CRE site.

An E-box overlaps the CRE site of the pgs2 gene and acts as an alternative site for binding of nuclear proteins (8). By adding E-box oligonucleotide competitor, we were able to reduce binding of proteins in nuclear extracts to the E-box sequence of the pgs2 promoter and enhance the binding of CREB and the additional nuclear factor(s) that binds to the PGS2 CRE site (8). The gel shift experiment shown in Fig. 2 was performed in the presence of E-box competitor to reduce (but not eliminate) the E-box complexes. The faster moving CRE site complex was previously identified, by supershift with anti-CREB antibody, as a CREB-containing complex (8). c-Jun antibody can recognize and supershift one of the slower moving complexes of the PGS2 CRE site (Fig. 2). In contrast, antibody to another protein (CREB binding protein) does not cause a supershift. c-Jun antibody alone does not form any complex with the CRE probe. The data demonstrate that the c-Jun protein is able to participate in a binding complex at the PGS2 CRE site.

v-src Activates JNK—Although the ERK1 and ERK2 MAP kinases can phosphorylate c-Jun in vitro (20), it is now clear that the recently cloned (21) JNKs are the kinases that generally phosphorylate and activate c-Jun in vivo (21–23). JNK1 and JNK2 are strongly activated by tumor necrosis factor α (24), environmental stress (24), and UV light (22). If c-Jun activation plays a role in v-src-mediated PGS2 induction, we would expect that v-src expression would activate JNK. Flag epitope-tagged JNK1 was immunoprecipitated, with an anti-Flag monoclonal antibody, from NIH 3T3 cells cotransfected either with a vector encoding Flag epitope-tagged JNK1 and either the v-src expression vector pMV-src or the empty vector pEVLX. Immunocomplex kinase assays were then performed, using a GST-c-Jun recombinant protein as substrate (15). Expression of v-src activates JNK1 kinase activity (Fig. 3).

The MEKK-1/JNK Signal Transduction Pathway Mediates v-src Induction of pgs2 Gene Expression—If v-src-mediated induction of PGS2 requires JNK activation and phosphorylation of c-Jun, we would expect that modulation of JNK levels in cells would alter the response of the pgs2 promoter to v-src expression. This is the case; overexpression of wild-type JNK1 potentiates v-src-mediated luciferase expression from the pTIS10-\beta\_Luc luciferase reporter gene (Fig. 4, left panel, lane 3), while co-expression of a kinase-defective, dominant-negative JNK1 attenuates v-src-mediated expression of luciferase from this reporter (Fig. 4, left panel, lanes 4 and 5). These data suggest that JNK activation plays an obligate role in v-src-mediated induction of gene expression from the pgs2 promoter.

We previously demonstrated that v-src-induced pgs2 gene expression required mediation by Ras (8). Ras activates several signal transduction pathways, each pathway leading to phosphorylation of distinct subsets of transcription factors (25). The downstream effector of Ras leading to activation of JNK enzyme activity and phosphorylation of c-Jun is the MAP kinase kinase MEKK-1 (15). In contrast, Ras activation of the Raf-1 MAP kinase kinase kinase leads to phosphorylation of transcription factors such as TCF/E1k-1 and c-Myc (25). Expression of kinase-defective, dominant-negative MEKK-1 blocks v-src-mediated expression of luciferase from the pTIS10-\beta\_Luc luciferase reporter gene (Fig. 4, right panel). We conclude that activated transcription from the pgs2 gene following expression of pp60\src requires Ras activation of MEKK-1 and JNK, leading to phosphorylation of c-Jun, and subsequent increased transcription mediated by the CRE site of the pgs2 promoter.

The c-Jun Activation Domain Is Required for v-src-mediated Luciferase Expression from a gal4-\_pgs2 Chimeric Promoter—We wished to more fully explore the nature of the regulated activation domain(s) required to drive luciferase expression by the transcription factor complex assembled at the pgs2 gene in response to pp60\src. We replaced the CRE sequence in the pgs2 luciferase reporter with yeast transcription factor GAL4.

**FIG. 2.** c-Jun binds to the CRE site of the pgs2 promoter. A 32P probe from nucleotides −65 to −39 of the pgs2 promoter was used for EMGS assay. E-box competitor (75-fold excess) was used in lanes 2-4 to reduce the contribution of the E-box complexes. The E-box binding complexes and the CRE binding complexes indicated in the figure have been identified previously, both by competition with consensus E-box and CRE sequences, and by EMGS-antibody supershift experiments (8). The c-Jun-containing complex is identified on the basis of the c-Jun antibody-dependent supershift.
Fig. 4. v-src activation of the pgs2 promoter is mediated by the MEKK1/JNK signal transduction pathway. Left panel, pTIS10-ΔpGAL4-LUC (3 µg) was cotransfected with the pp60v-src expression vector pMV-src (1.5 µg) and with expression vectors encoding wild-type JNK (2 µg) or kinase-defective JNK (2 and 4 µg), as shown in the figure. DNA concentrations for transfection were held constant by including DNAs for appropriate empty expression vectors. Right panel, pTIS10-ΔpGAL4-LUC (3 µg) was cotransfected with the pp60v-src expression vector pMV-src (1.5 µg) and increasing amounts of dominant-negative MEKK-1 expression vector. The total amount of DNA in each transfection was kept constant by using appropriate empty expression vectors. Three plates were used for each transfection condition. Data are expressed as averages ± S.D.

DNA binding sites (Fig. 5, top) and investigated what transcription domains in chimeric GAL4 DNA binding proteins can mediate v-src activation of the minimal pgs2 promoter. No endogenous transcription factor in 3T3 cells will bind to the GAL4 sites.

A luciferase reporter gene containing only the first 40 nucleotides of the pgs2 promoter, T1S10-ΔpGAL4-LUC, expresses only minimal luciferase activity. This plasmid does not contain the overlapping CRE and E-box sites of the pgs2 promoter and is not responsive to v-src expression (8). If five GAL4 binding sites are added to this promoter, this gal4-pgs2 luciferase construct similarly expresses only minimal luciferase activity (Fig. 5, middle panel, lane 1) and does not respond to v-src expression (Fig. 5, middle panel, lane 3). Expression of the GAL4 DNA binding domain alone (GAL4-DB) does not enhance luciferase expression from the gal4-pgs2 luciferase chimeric reporter vector (Fig. 5, middle panel, lane 2; lower panel, lane 1) or mediate v-src induction from this reporter (Fig. 5, middle panel, lane 4).

Expression of a fusion protein containing the GAL4 DNA binding domain and the c-Jun activation domain (amino acids 5–200; GAL4-c-Jun) can activate basal expression from the Gal4-T1S10-ΔpGAL4-Luciferase chimeric reporter vector (Fig. 5, middle panel, lane 5; lower panel, lane 2). However, when pp60v-src is also expressed, along with the GAL4-c-Jun protein, expression from the Gal4-T1S10-ΔpGAL4-Luciferase reporter vector is substantially enhanced (Fig. 5, middle panel, lane 6; lower panel, lane 3). We conclude that the c-Jun activation domain, if positioned upstream of the minimal pgs2 promoter, can drive v-src-induced gene expression. In contrast, if the activation domains of ATF-2 or CREB are positioned adjacent to the minimal pgs2 promoter by the GAL4 DNA binding domain of GAL4-DB chimeric proteins, these activation domains are unable to mediate either basal or v-src-induced luciferase expression from the pgs2 promoter (Fig. 5, lower panel, lanes 5 and 6).

Phosphorylation of two serine residues, Ser-63 and Ser-73, is required for c-Jun activation and transcription from AP-1 sites (26, 27). To determine if phosphorylation of these two sites is essential for v-src-induced expression from the pgs2 reporter, we used an expression vector in which the serines at these two sites have been mutated to leucines. GAL4-c-Jun 63/73, in which these sites of phosphorylation have been altered, is unable to mediate v-src activation of the gal4-pgs2 luciferase reporter gene (Fig. 5, lower panel, lane 4). An intact c-Jun activation domain, with sites of phosphorylation available, is necessary for v-src-mediated transcriptional activation of the pgs2 promoter.

ERK-1 and ERK-2 Activation Also Participate in PGS2 Induction by v-src—Like JNK, the MAP kinase enzymes ERK-1 and ERK-2 can also be activated by pp60v-src expression (28, 29). We therefore asked whether these kinases might also mediate PGS2 induction by v-src. Co-expression of ERK-1 or ERK-2 potentiates v-src-mediated luciferase expression from the pTIS10-ΔpGAL4-Luciferase reporter gene (Fig. 6, left panel). Moreover, co-expression of kinase-deficient, dominant-negative forms of ERK-1 or ERK-2 substantially attenuates v-src-mediated luciferase expression from this reporter.

Raf-1 is the MAP kinase kinase kinase that mediates activation of the ERK enzymes by Ras (25). If the Ras/Raf-1/MEK/ERK pathway plays a role in v-src induction of gene expression from the pgs2 promoter, one would expect that a dominant-negative Raf-1 mutation should also block this induction. This is the case; increased inhibition of v-src-mediated luciferase induction from the pgs2 promoter is observed as increasing amounts of a dominant-negative Raf-1 protein are expressed.

Fig. 5. The c-Jun activation domain is required for v-src-mediated luciferase expression from the pGal4TIS10-ΔpGAL4-LUC chimeric promoter. Top panel, the reporter construct pGal4TIS10-ΔpGAL4-LUC, consisting of five GAL4 DNA binding sites ligated to nucleotides −40 to +3 of the tis10/pgs2 promoter and driving the luciferase reporter gene. Middle panel, pGal4TIS10-ΔpGAL4-LUC (3 µg) was cotransfected with 1 µg of the vectors expressing either the GAL4 DNA binding domain (GAL4-DB) or the GAL4-DB fused to the c-Jun activation domain (GAL4-c-Jun). Lower panel, 3 µg of pGal4TIS10-ΔpGAL4-LUC was cotransfected with 1 µg of the vectors expressing either GAL4-DB or GAL4-DB fused to the indicated transcription factors. Three plates were used for each transfection condition. Data are expressed as averages ± S.D.
The c-Jun transactivation domain, when fused to a GAL4 DNA binding domain, can drive expression from a pgs2 promoter in which the CRE is replaced by GAL4 DNA binding sites. In contrast, ATF transactivation domains placed at this site are unable to mediate pgs2 gene transcription. Therefore, two components of the AP-1 transcription complex may participate in the v-src activated transcription of the pgs2 promoter from the CRE site.

**FIG. 6.** Activation of ERK-1 and/or ERK-2 is also required for PGS2 induction by v-src. Left panel, pTIS10−gal-Luc (3 µg) was cotransfected with the pMV-src pp60v-src expression vector (1.5 µg) and with expression vectors encoding wild-type ERK-1 or ERK-2 proteins (1 µg) or with expression vectors encoding dominant-negative ERK-1 or ERK-2 proteins (2 µg). Right panel, pTIS10−gal-Luc (3 µg) was cotransfected with the pMV-src pp60v-src expression vector (1.5 µg) and increasing amounts of the expression vector encoding a dominant-negative Raf-1 protein. The total amount of DNA in each reaction was kept constant by using corresponding empty expression vectors. Three plates were used for each transfection condition. Data are expressed as averages ± S.D.

**FIG. 7.** Overexpression of c-jun and c-Fos augments PGS2 induction by v-src. Cells were transfected with the v-src expression vector pMV-src or the empty vector pEVX, along with the pTIS10−gal-Luc luciferase expression vector and expression vectors for c-jun (2 µg), c-Fos (2 µg), ATF-3 (2 µg), CREB (2 µg), or a combination of c-jun and c-Fos (1 µg each). The total amount of DNA in each transfection was kept constant by using corresponding empty expression vectors. Three plates were used for each transfection condition. Data are expressed as averages ± S.D.

**DISCUSSION**

Elevated prostaglandin production is a characteristic of Rous sarcoma virus-transformed fibroblasts (30). Persistent elevation of pgs2 gene expression in v-src-transformed cells is responsible for this increased prostaglandin production (3, 31). A CRE at nucleotides −56 to −52 of the pgs2 promoter is the critical element in v-src-mediated activation of the pgs2 gene (8). pp60v-src has been reported to modulate immediate-early gene expression through the serum response element of the egr1/tis8 gene (32), at a dyad symmetry element and a Sis-inducible factor responsive element in the c-fos gene (33), through the CCAAT and TATAA elements of the junB gene (34), and via a v-src-responsive element of the 9E3/CEF-4 gene (35). However, v-src modulation of gene expression via a CRE has only been observed for the pgs2 gene (8).

**c-jun Transcriptional Activation Plays a Major Role in v-src-induced pgs2 Gene Expression**—We anticipated that the CRE-dependent v-src transactivation of the pgs2 gene might involve c-jun activation, since oncogenic forms of both Ras and Src can activate transcription via c-jun phosphorylation (26, 27). Our demonstration that v-src-induced activation of JNK activity correlates with PGS2 induction is consistent with this hypothesis. The ability of dominant-negative inhibitory forms of Ras, MEKK, and JNK, the mediators of the c-jun phosphorylation/activation pathway, to block v-src-induced expression from the pgs2 promoter also supports this proposal. The antibody supershift demonstration that c-jun is a part of a protein complex that can recognize the PGS2 CRE also supports this hypothesis. Based on these data, we conclude that v-src activates the Ras/MEKK/JNK signal transduction pathway to phosphorylate c-jun and stimulate transcription from the CRE of the pgs2 gene, v-src-mediated activation of c-jun and pgs2 gene transcription is diagramed in Fig. 8.

The c-jun transactivation domain, when fused to a GAL4 DNA binding domain, can drive expression from a pgs2 promoter in which the CRE is replaced by GAL4 DNA binding sites. In contrast, ATF transactivation domains placed at this site are unable to mediate pgs2 gene expression. Moreover, this response is eliminated when the sites of JNK phosphorylation are mutated in the c-jun transactivation domain of the GAL4-JUN chimeric transactivator, clearly demonstrating that phosphorylation of the c-jun transactivation domain by JNK plays a critical role in v-src-induced transcription from the pgs2 gene.

**c-jun is also phosphorylated following exposure of cells to inflammatory mediators such as tumor necrosis factor a (24) and interleukin 1 (21).** Tumor necrosis factor a and interleukin 1 are also potent activators of prostaglandin production and induce the expression of the pgs2 gene in rat mesangial cells (38). Thus, c-jun activation and the CRE of the pgs2 promoter may also play a role in mediating PGS2 induction by these inflammatory cytokines. UV irradiation is among the strongest JNK activators (21). It will be of great interest to determine whether UV radiation can induce pgs2 gene expression.

**ATF-2 Does Not Mediate v-src-induced pgs2 Gene Expres-
nase activity on v-src-mediated induction of pg2 gene expression, once this kinase(s) has been cloned, to determine if direct activation of c-Fos plays a role in this v-src-induced response. We suggest that v-src may be a powerful activator of immediately-early gene transcription because it can activate a variety of kinase-mediated signal transduction cascades, leading to phosphorylation and transcriptional activation of a number of pre-existing transcription factors.

The Ras/Raf/ERK Pathway Plays a Role in v-src-induced pg2 Gene Expression—v-src-activated expression from the PGS2 CRE can be enhanced by expression of ERK proteins and partially blocked by expression of dominant interfering ERK or Raf-1 proteins. These results demonstrate that the Raf/MEK/ERK (MAPK) pathway also plays a role in v-src activation of pg2 gene expression at the PGS2 CRE. ERKs do not transcriptionally activate either c-Jun or c-Fos by direct phosphorylation (23, 25). They do, however, increase AP-1 activity in cells by phosphorylating and activating the transcription factor TCF/Elk-1 responsible for increased expression from the c-Fos gene (23, 25). One might expect that the Raf/MEK/ERK pathway might play a major role in secondary response genes whose induction is mediated by AP-1, following the immediate-early gene mechanism that increases c-Fos and c-Jun proteins in cells. In this case, protein X expressed in response to MAPK activation of ELK/SRF in Fig. 8 would be the c-Fos protein. In this regard, it is of considerable interest to note that PGS2 expression following v-src activation is persistent; pg2 expression remains constantly elevated in v-src-transformed cells (3, 30). In contrast, PGS2 induction by growth factors, tumor promoters, or inflammatory cytokines is transient and returns to basal values even if ligand is present continuously (6). These data suggest that v-src may induce AP-1 expression by a Raf/MEK/ERK pathway, activating an additional, transcription-dependent mechanism of increased pg2 gene expression that is not shared with other subunits.

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