c-Src-dependent Transcriptional Activation of TFII-I*

Received for publication, March 27, 2002, and in revised form, April 3, 2002
Published, JBC Papers in Press, April 4, 2002, DOI 10.1074/jbc.M202956200

Venugopalan Cheriyath‡, Zana Patrick Desgranges§, and Ananda L. Roy¶

From the ‡Department of Pathology and Programs in §Immunology and ¶Genetics, Tufts University School of Medicine, Boston, Massachusetts 02111

TFII-I is a multifunctional transcription factor that is also involved in signal transduction. Here we show that TFII-I undergoes a c-Src-dependent tyrosine phosphorylation on tyrosine residues 248 and 611 and translocates to the nucleus in response to growth factor signaling. Tyrosine-phosphorylated nuclear TFII-I activates a stably integrated c-fos reporter gene. Withdrawal of signal leads to diminution of nuclear TFII-I, suggesting that the signal-dependent translocation is reversible. Antibodies against either TFII-I or c-Src abrogate growth factor-stimulated activation of c-fos. Consistent with the notion that tyrosine phosphorylation of TFII-I is required for its transcriptional activity, phosphorylation-deficient mutants of TFII-I fail to activate the c-fos promoter. These data demonstrate that TFII-I, through a Src-dependent mechanism, reversibly translocates from the cytoplasm to the nucleus, leading to the transcriptional activation of growth-regulated genes.

Extracellular signals are ultimately transduced to the nucleus through a series of complicated biochemical steps that result in the activation of specific genes. Inducible transcription factors often play a critical role in this process by responding to the cell's external signals. Here we demonstrate that the multifunctional transcription factor TFII-I is activated in response to extracellular signals, translocates into the nucleus, and thus links signal transduction events to transcription.

Based on its unique interactions at both a core promoter element and upstream regulatory sites, TFII-I is postulated to be a novel transcription factor that facilitates communication between upstream regulatory proteins and the basal machinery (1–3). There are four alternatively spliced isoforms of TFII-I, all of which are characterized by the presence of six I repeats, R1–R6, each containing a potential helix-loop-helix motif implicated in protein-protein interactions (4, 5). Recent genetic and biochemical data also indicate that TFII-I belongs to a family of protein characterized by the presence of I-repeat, first identified in TFII-I (6). Besides its transcription functions, TFII-I is shown to be phosphorylated at both serine/threonine and tyrosine residues, and tyrosine phosphorylation is critical for its transcriptional activity (7). Furthermore, it has been shown that a variety of growth-related signals lead to enhanced tyrosine phosphorylation and increased transcriptional activity of TFII-I (7, 8). In the B-cell cytoplasm, a large fraction of TFII-I is associated constitutively with Bruton's tyrosine kinase (9, 10). TFII-I is tyrosine-phosphorylated by Bruton's tyrosine kinase in vitro (10), and upon immunoglobulin receptor cross-linking in B cells, TFII-I is released from Bruton's tyrosine kinase to enter the nucleus (9). Thus, mutations impairing the association between TFII-I and Bruton's tyrosine kinase may result in improper TFII-I localization, activation, and diminished transcription, leading to defective B-cell function (9).

Whereas TFII-I appears to be localized largely in the cytoplasm of a variety of untransformed cells, it is constitutively localized in the nucleus of transformed cells. Moreover, the “basal” tyrosine phosphorylation (in the absence of extracellular signals) of TFII-I is comparatively higher in transformed cells than in primary cells, suggesting that both the tyrosine phosphorylation and nuclear localization of TFII-I may be deregulated during transformation. We demonstrate herein that in untransformed fibroblasts, the non-receptor tyrosine kinase c-Src controls both the tyrosine phosphorylation and the nuclear localization of TFII-I upon growth factor signaling. The major Src-dependent tyrosine phosphorylation sites of TFII-I are also required for its transcriptional activity. Tyrosine-phosphorylated nuclear TFII-I activates a chromosomally integrated c-Fos reporter gene in vivo. However, TFII-I is constitutively located in a non-tyrosine-phosphorylated form in the nucleus of cells lacking Src family kinases. Hence, TFII-I appears to be tethered to the cytoplasm of untransformed fibroblasts in a Src-dependent fashion. Consequently, in the absence of Src, TFII-I is not tethered to the cytoplasm and found constitutively in the nucleus. We conclude that the tyrosine phosphorylation status, nuclear translocation, and the transcriptional activity of nuclear TFII-I are c-Src-dependent.

EXPERIMENTAL PROCEDURES

Cell Lines and Cell Culture—NIH 3T3, COS7, HA-13 (11), and SYF (American Type Culture Collection) cells were grown in Dulbecco's modified Eagle's medium (Invitrogen) containing 10% calf serum (Invitrogen), 50 units of penicillin, and 50 μg/ml streptomycin (Invitrogen) at 37 °C under 5% CO₂. The NIH 3T3 cell line stably integrated with dominant negative Src was grown in the above-mentioned media supplemented with G418 (600 μg/ml).

Plasmids—The construction of PEBGII-I wild type and Y248F mutant has been described previously (7, 12). The expression plasmid encoding the wild-type or dominant negative c-Src has been described previously (13). PCR-based mutagenesis was used to create an additional mutation at the Tyr621 residue (Y-F2).

Antibodies—The antibodies used were as follows: anti-TFII-I antibody (12), anti-c-Src antibody (GD11 clone; Upstate Biotechnology), and anti-c-Src antibody (BC-12; Santa Cruz Biotechnology). An IgG-purified anti-TFII-I antibody was used in immunostaining (4). The anti-P-TFII-I antibody was raised against the phosphopeptide SEDPD[pY]YQYNI and subsequently affinity-purified (Research Genetics).

Generation of a Stable NIH 3T3 Cell Line—The stable cell line expressing dominant negative Src was derived as described previously (13), with the following modifications. One day before transfection, the 80–90% confluent cells were split 1:5 and transfected with 10 μg of the plasmid RC-CMV-Src containing dominant negative Src cDNA. Transfection was carried out with superfect reagent (Qiagen). Neo-integrated

* This work was supported by National Institutes of Health Grant AI45150 (to A. L. R.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡To whom correspondence should be addressed: Dept. of Pathology and Programs in Immunology and Genetics, Tufts University School of Medicine, 136 Harrison Ave., Boston, MA 02111. Tel.: 617-636-6715; Fax: 17-636-2990; E-mail: ananda.roy@tufts.edu.

80–90% confluent cells were split 1:5 and transfected with 10 μg of the plasmid RC-CMV-Src containing dominant negative Src cDNA. Transfection was carried out with superfect reagent (Qiagen). Neo-integrated
cells were selected against G418 and subcloned to establish stable clones expressing dominant negative Src.

**Transfection of COS7 Cells**—COS7 cells were transfected with 7.5 μg of wild-type or mutant TFII-I expression plasmids (pEBGI-I, pEBGI-I F1, and pEBGI-I Y-F2) with or without 7.5 μg of wild-type or dominant negative c-Src by lipofection (4). Epidermal growth factor (EGF)1 (25 ng/ml; Sigma) stimulation of the cells was carried out as described previously (4).

**Antibody Transfection**—HA-13 cells were transfected with preimmune serum or specific antibodies using the CHARIOT reagent (Active Motif). Where indicated, the cells were stimulated with platelet-derived growth factor (PDGF; 25 ng/ml) and fixed at different time points.

**Reportor Assays**—COS cells were transfected with 600 ng of c-fos-luciferase reporter plasmid with either pEGB vector, wild-type TFII-I, or its mutants (pEBGI-I, pEBGI-I Y-F1, and pEBGI-I Y-F2) in the absence or presence of c-Src (RC-CMV-Src) as described previously (5). 24 h after transfection, the cells were serum-starved for 12 h and then stimulated with EGF for 4 h. The luciferase activities were then determined (Dual Luciferase Assay; Promega Corp).

**GST Pull-down Assay**—Whole cell extract (200 μg) from COS7 cells transfected with wild-type or mutant TFII-I with or without c-Src were subjected to GST pull-down (35 μl; 1:1 slurry; Sigma) as described previously (4). The blots were probed with anti-TFII-I antibody (1: 2500), anti-P-Tyr antibody (1:2000; Santa Cruz Biotechnology), or anti-HA (1:1000; cocodyl)- conjugated antibody.

**Western Blot Analysis**—For Western blot analysis, the primary anti-TFII-I (1:2500 dilution) and anti-c-Src (1:1000 dilution) antibodies and the secondary anti-rabbit horseradish peroxidase-linked (1:10,000 dilution) antibody (Zymed Laboratories Inc.) were incubated in Tris-buffered saline containing 0.05% Tween 20. All Western blots were developed using a Renaissance kit (PerkinElmer Life Sciences).

**Immunostaining**—The cells were grown on coverslips, serum-starved for 18–20 h, and stimulated with PDGF (20 ng/ml). 10 min after stimulation, the cells were washed three times with Dulbecco’s modified Eagle’s medium and fixed in methanol at various time points. The cells were incubated with primary antibodies (anti-TFII-I, 1:1000; anti-P-TFII-I, 1:5000; or anti-FA (12CA5), 1:1000) and with secondary antibody (anti-rabbit IgG conjugated with Alexa 488 or with anti-rabbit IgG Alexa 594) at a dilution of 1:10,000 for 1 h. The cells were finally covered with mounting buffer (90% glycerol with 0.02% sodium azide).

## RESULTS

**TFII-I Contains Several Src-dependent Functional Tyrosine Phosphorylation Sites**—The presence of two consensus Src tyrosine phosphorylation sites (EDSDK at amino acid positions 244–248 and 273–277, respectively) prompted us to test whether TFII-I undergoes a Src-dependent tyrosine phosphorylation and whether that might control the transcriptional activity of TFII-I. In addition to those consensus tyrosine phosphorylation sites, two other YXXMP motifs (amino acids 373–376 and 611–614) were also identified that are known to bind to Src homology 2 domains (14). To test the functionality of these tyrosine residues, we generated several (tyrosine-phenylalanine) mutants of TFII-I. Of these, the Y-F277 and Y373 mutants did not show appreciable effects in any assays tested and were therefore not pursued (data not shown). Y-F248 (Y-F1) and the double mutant Y-F248 + Y-F611 (Y-F2) were tested for Src-dependent phosphorylation and transcriptional assays. Wild-type TFII-I or mutant TFII-I proteins were expressed in COS7 cells as GST fusion proteins in the absence or presence of ectopic c-Src, and their tyrosine phosphorylation status was tested (Fig. 1A). Whereas the basal tyrosine phosphorylation of wild-type TFII-I in the absence of ectopic c-Src was barely detectable (Fig. 1A, top panel), co-expression of c-Src significantly increased the tyrosine phosphorylation of TFII-I. In contrast, there was a marked decrease in the tyrosine phosphorylation of both Y-F1 and Y-F2 mutants. This experiment was repeated three times, and the results were plotted. The Y-F1 mutant showed nearly 60% reduction, whereas the Y-F2 mutant showed >80% reduction in tyrosine phosphorylation compared with wild-type TFII-I (Fig. 1B). We previously showed enhanced tyrosine phosphorylation of TFII-I in response to EGF treatment (8). It is also known that EGF stimulation leads to activation of Src (15). Whereas EGF stimulation significantly increased tyrosine phosphorylation of wild-type TFII-I (Fig. 1C), the Y-F1 mutant exhibited an 8-fold decrease in tyrosine phosphorylation, suggesting that EGF-dependent induction of tyrosine phosphorylation in TFII-I also occurs at Tyr248. The Y-F1 and Y-F2 mutants were further tested in transcription assays. Ectopic expression of c-Src alone did not significantly increase the c-fos promoter activity, but when it was expressed together with ectopic TFII-I, promoter activity was enhanced (Fig. 1D). The transcriptional activity achieved under these conditions was comparable to that obtained upon EGF stimulation (data not shown). Most importantly, co-expression of Src and either the Y-F1 or Y-F2 mutant failed to significantly increase c-fos promoter activity over the basal levels. Whereas the basal level expression of Y-F1 (Fig. 1D, lane 5) was lower than that of the wild type (lane 3), their levels of expression were very similar in the presence of Src (lane 4 versus lane 6). Hence, the Src-dependent tyrosine phosphorylation sites in TFII-I are functional and required for its c-fos-dependent transcriptional activity.

**Signal-dependent Nuclear Translocation of Endogenous TFII-I**—Regulation by Endogenous Src—Given that TFII-I undergoes Src-dependent tyrosine phosphorylation and that Src is a cytoplasmic kinase, we tested whether TFII-I is cytoplasmic in normal NIH 3T3 cells and, if so, whether the subcellular localization of endogenous TFII-I is altered upon stimulation in a Src-dependent fashion. Because expression of the EGF receptor is limited in NIH 3T3 cells (16), we used PDGF as the source of growth factor. In the absence of PDGF, the majority of endogenous TFII-I was extranuclear (with diffuse nuclear staining; Fig. 2A, −PDGF). In contrast, upon PDGF stimulation, the majority of TFII-I staining was observed in the nucleus (Fig. 2A, +PDGF). The loss of Src dependence, cells were treated with either a Src-specific inhibitor, PP1, or with its noninhibitory analogue, PP3 (17). Pretreatment of cells with PP1 significantly decreased signal-induced nuclear translocation of TFII-I (Fig. 2A, +PDGF+PP1), whereas its analogue, PP3, had no effect (Fig. 2A, +PDGF−PP3). Thus, signal-induced nuclear translocation of TFII-I is likely a Src-dependent process. We then established an NIH 3T3 cell line that expresses a dominant negative c-Src and tested the localization of TFII-I in these cells and in SYF cells in which Src family kinase members Src, Yes, and Fyn were all genetically deleted (18). The majority of TFII-I localized constitutively in the nucleus of cells expressing dominant negative c-Src and SYF cells (Fig. 2A, NIH-3T3(dn) and SYF). TFII-I subcellular localization remained unchanged even in the presence of PDGF in dominant negative Src-expressing cells and SYF cells (data not shown), although it remains to be seen whether c-fos is activated in response to PDGF in these cells. In summary, we conclude that Src or a Src family kinase directly or indirectly tethers TFII-I to the cytoplasm, such that inactivating Src or genetically deleting Src family kinases leads to constitutive nuclear localization of TFII-I. Importantly, the nuclear translocation of TFII-I in normal NIH3T3 cells was reversible because withdrawal of PDGF led to significant reduction in nuclear TFII-I staining together with a concomitant increase in extranuclear TFII-I (Fig. 2B).

**Signal-induced Nuclear Translocation of Endogenous TFII-I Correlates with Expression of Integrated c-fos**—To address the

---

1 The abbreviations used are: EGF, epidermal growth factor; PDGF, platelet-derived growth factor; GST, glutathione S-transferase; DAPI, 4′,6-diamidino-2-phenylindole; SRE, serum response element; HA, hemagglutinin; WBS, Williams Beurens syndrome.
transcriptional consequences of signal-induced nuclear translocation of TFII-I, we used cell line SRE-Fos HA (clone 13), which contains a stably integrated c-fos gene tagged internally with HA under the SRE promoter. Because the ternary complex factors cannot associate with this promoter (11, 19), and TFII-I can bind to the sites overlapping the SRE (8, 20), activation of such a promoter may reflect TFII-I dependence. Stimulation with PDGF led to significant nuclear translocation of TFII-I (Fig. 3, compare E–G, and H) and the concomitant appearance of nuclear HA-Fos (Fig. 3F). Accumulation of nuclear TFII-I continued for an additional 30 min. Peak HA activity was observed between 10 and 20 min of PDGF withdrawal (compare Fig. 3, I–L with M–P). By 60 min, most of the TFII-I was extranuclear, with a simultaneous reduction of HA staining. Thus, a good correlation exists between the kinetics of nuclear localization of TFII-I and the expression of HA-Fos.

To demonstrate a direct role of signal-induced nuclear TFII-I in the regulation of HA-Fos transcription, we transfected either preimmune sera or an anti-TFII-I antibody into HA-13 cells using a lipid-mediated method (21). The introduction of preimmune serum into these cells did not abolish HA expression, and maximum HA-Fos expression was observed between 10 and 20 min.
min after PDGF withdrawal (Fig. 4, J and N). Most significantly, transfection of either an anti-TFII-I antibody or an anti-Src antibody abrogated HA-Fos expression, suggesting that PDGF-induced activation of HA-Fos is dependent on both TFII-I and c-Src (Fig. 4, A/ H11032/H11002 X/H11032).

Signal-induced Nuclear TFII-I Is Tyrosine-phosphorylated at Tyr248—Using ectopically expressed TFII-I, we showed that Tyr248 exhibits enhanced tyrosine phosphorylation in response to growth factor stimulation and when co-expressed with ectopic Src. Moreover, we demonstrated that tyrosine phosphorylation of Tyr248 is critical for transcriptional activity of TFII-I. However, it was not known whether this site was also tyrosine-phosphorylated in endogenous TFII-I. To demonstrate that Tyr248 is tyrosine-phosphorylated in vivo, we generated a phospho-specific antibody against phosphorylated Tyr248. To ensure specificity, we first tested this antibody against ectopically expressed TFII-I and its mutants in COS7 cells (Fig. 5A). The antibody recognized only the wild-type TFII-I in the presence of EGF (Fig. 5A, lane 2). This recognition was completely lost in the presence of the Src inhibitor PP2 (lane 4; PP2 was used instead of PP1 because PP1 interferes with the EGF receptor) or when Tyr248 was mutated as in Y-F1 (lane 6) and Y-F2 (lane 8). Moreover, this antibody stained predominantly nuclear TFII-I upon stimulation, although a diffuse staining could be observed at earlier time points (Fig. 5B). Importantly, the time course of tyrosine phosphorylation correlated with the time course of nuclear translocation and HA-Fos expression (see Fig. 3). Moreover, tyrosine phosphorylation of TFII-I at Tyr248 was not observed in SYF cells, although TFII-I is constitutively present in the nucleus of these cells. Therefore, Tyr248 tyrosine phosphorylation of TFII-I is Src family kinase-dependent. Although it was reported recently that JAK2 phosphorylates TFII-I at Tyr248 (22), in our hands, TFII-I is tyrosine-phosphorylated on Tyr248 and translocates to the nucleus in JAK2-null fibroblasts (23), suggesting that these processes are JAK2-independent (data not shown). However, it is possible that under some conditions and using some assays, JAK2-dependent phosphorylation of TFII-I may be seen.

**DISCUSSION**

Src family protein tyrosine kinases are activated following the engagement of diverse cellular receptors, thereby partici-
pating in altering various biological responses including cell proliferation, migration, differentiation, and survival (24). Src is perhaps the best-studied non-receptor protein tyrosine kinase involved in regulating cellular responses to various extracellular stimuli (25, 26). It is the first defined proto-oncogene, and the viral form of cellular Src (v-Src) encodes a constitutively active enzyme that can induce cellular transformation (27).

Triggering of growth factor receptors via their cognate ligands (e.g. PDGF and EGF) in fibroblasts leads to activation of c-Src through its autophosphorylation, and several reports, using biochemical evidence, suggest that Src family kinases are essential components of PDGF receptor signaling (28–32). However, PDGF receptor-mediated signaling and mitogenesis remain intact in fibroblasts derived from mice with targeted disruption of three Src family kinases (Src, Yes, and Fyn) (18). One interpretation of these results is that activation of Src family protein tyrosine kinases is dispensable, at least in some instances and cell types, for PDGF-driven mitogenesis and that the SYF (Src, Yes, Fyn) triple mutant phenotype is not likely to be caused by abnormal PDGF receptor signaling (18). Alternatively, it can be argued that PDGF receptors activate many parallel signaling events, each of which can lead to mitogenesis (32). Hence, it is very likely that a requirement for Src family kinases in SYF cells is bypassed by activation of any of these PDGF receptor-mediated signaling pathways involving intermediates such as phosphatidylinositol 3-kinase or phospholipase C (18).

Numerous proteins interact with and are phosphorylated by c-Src (24), but they do not provide mechanistic insight as to how activated Src might regulate growth factor-dependent gene expression (33, 34). Moreover, although the oncogenic form of Src activates signal transducers and activators of transcription, a direct physical link has not been established (35). It has also been shown that integrin signaling through the Src family kinase Fyn leads to recruitment of the Shc adapter protein, resulting in activation of an artificial SRE-fos reporter via the Ras-mitogen-activated protein kinase pathway (36). Adding to this complexity is the fact that knockouts of c-Src resulted in a minimal phenotype, presumably due to functional redundancy in the Src family kinases (37). Consistent with this idea, double and triple knockout (SYF) mice exhibit a severe

FIG. 3. Signal-induced nuclear localization of TFII-I correlates with c-fos expression. Double immunostaining of HA-13 cells was performed with anti-TFII-I followed by Alexa 488 secondary antibody (green), with anti-HA followed by Alexa 594 secondary antibody (red) for HA-Fos expression, or with DAPI (blue) for nuclei staining. Time course: unstimulated (0 min), A – D; 10 min after stimulation with PDGF, E – H; 10 min after PDGF withdrawal, I – L; 20 min after PDGF withdrawal, M – P; 40 min after PDGF withdrawal, Q – T; and 1 h after PDGF withdrawal, U – X.
phenotype, including embryonic lethality (18). In contrast, Src\(^{-/-}\) mice exhibit osteopetrosis with decreased absorption of bone, resulting in bone overgrowth (37, 38). Furthermore, transgenic studies suggest that Src has a kinase-independent function, particularly in osteoclasts (38). Although osteoclasts may represent a special cell type because the levels of Src expression in osteoclasts are unusually high, it is likely that Src may be important for regulating the localization of certain proteins or stabilizing signaling complexes that control cellular growth in other cell types as well (26). Collectively, these data suggest that all Src-dependent signals are not transduced through a single pathway. Consistent with the notion of a novel Src-dependent pathway, we postulate that Src controls tyrosine phosphorylation and subsequent nuclear translocation of TFII-I, resulting in up-regulation of growth-promoting genes. However, the precise mechanism of how Src controls nuclear translocation and tyrosine phosphorylation of TFII-I remains to be determined. Currently, we cannot formally rule out the possibility that TFII-I may not be a direct substrate of Src. Moreover, Src is localized predominantly in the membrane, whereas extranuclear TFII-I is most likely in the soluble fraction. Although a fraction of TFII-I is constitutively associated with Src under normal conditions (data not shown), this may not be sufficient to physically tether the majority of cellular TFII-I to the cytoplasm. It is conceivable that other factors are involved in this pathway, and the identification of these factors may lead to a better understanding of how Src controls gene expression via TFII-I.

Regardless of the exact mechanism of Src-mediated regulation of TFII-I, it is clear that upon signaling, TFII-I rapidly becomes tyrosine-phosphorylated and translocates to the nucleus to activate various signal-induced genes, a process that requires an active Src. This process is completely reversible, thus providing a rapid turn on/off mechanism that is a prerequisite for controlled signal-induced cellular growth. It is also particularly gratifying to observe that the kinetics of TFII-I tyrosine phosphorylation and its concomitant nuclear import match the kinetics of HA-Fos gene expression, lending further credence to the notion that TFII-I is required for HA-Fos transcription. However, other investigators using the same HA-Fos cell line that we used in our system have shown that regulated association of Src with Diaphanous-related formins (mDia1 and mDia2) controls HA-Fos via activation of SRF (39). Our results are not incompatible with these observations and might suggest a coordinate regulation of HA-Fos via the activation of both TFII-I and SRF. In this regard, it is worth pointing out that the TFII-I binding site overlaps the SRF binding site (SRE), and the two proteins interact both on and off the DNA (8, 20). We also wish to emphasize that the Src-TFII-I pathway may have broader implications beyond PDGF-mediated up-

![Activation of TFII-I](https://www.jbc.org/)

**Fig. 4.** TFII-I or Src antibody abrogated PDGF-induced c-fos expression in HA-13 cells. HA-13 cells were transfected with preimmune sera (left panels, A–X), anti-TFII-I (A'–V'), or anti-Src (C'–X') by using CHARIOT reagent. The time course is the same as that described in the Fig. 3 legend. HA-Fos expression was determined by anti-HA antibody (B, F, J, N, R, and V). Nuclei were stained with DAPI (C, G, K, O, S, and W). The images were merged (Merge) (D, H, L, P, T, and X). Alexa 488 secondary antibody was used to detect either the preimmune or the anti-TFII-I (A', E', I', M', Q', and U') or the anti-Src-transfected cells (C', G', K', O', S', and W'). This was followed by immunostaining with anti-HA and Alexa 594 for anti-TFII-I transfected cells (B', F', J', N', R', and V') or anti-Src-transfected cells (D', H', L', P', T', and X').
regulation of c-fos. This is especially true because TFII-I is a multifunctional transcription factor that likely controls a wide variety of genes (6). Furthermore, it is important to note that this pathway may not be commonly utilized by all growth factors for their growth regulatory and transforming potentials.

Could there be a correlation between the Src-mediated activation of TFII-I and neurodevelopmental disorders observed in Williams Beurens syndrome (WBS)? This is particularly tantalizing because the highest expression of TFII-I is found in the brain, and there appears to be a neuron-specific isoform of TFII-I (1, 40). WBS is a multisystem dysfunction manifested as mild to moderate mental retardation, cognitive defects, and supravalvar aortic stenosis (41). Although it is likely that the broad phenotypic spectrum associated with WBS is the consequence of multigene deletion, the haploinsufficiency of TFII-I and its related gene suggests a potential link between TFII-I function and one or more of the WBS phenotypes (40). Perhaps

**FIG. 5. PDGF-induced phosphorylation of TFII-I at Tyr<sup>248</sup> in wild type but not in SYF cells.**

A. Lanes: GST alone, wild-type TFII-I without or with EGF, wild-type TFII-I with EGF plus PP2, wild-type TFII-I with Y-F1 mutant without or with EGF, and wild-type TFII-I with Y-F2 mutant without or with EGF. All ectopically expressed proteins were GST-tagged. B, tyrosine phosphorylation of endogenous TFII-I in NIH 3T3 cells (A–R) compared with SYF cells (A’–L’). The nuclei were stained with DAPI (B, E, H, K, N, and Q), and images were merged (C, F, I, L, O, R, B’, D’, F’, H’, J’, and L’).
a mouse knockout model in the near future might address a potential connection between Src-mediated activation of TFII-I and cognitive defects associated with WBS.

Acknowledgments—We are grateful to Larry Feig for critical reading of the manuscript. We are grateful to Joan Brugge and Larry Feig for providing the wild-type and dominant negative Src constructs and to Jim Ihle, Nick Carpino, and Evan Parganas for the JAK2 knockout fibroblasts. We especially thank Art Alberts for his generosity in providing the HA-13 cell line. Finally, we thank past and present laboratory members including Changchuin Mao, Ashit Dube, Carl Novina, Catarina Sacristan, and Isabel Tusie-Luna for their help.

REFERENCES
1. Roy, A. L., Du, H., Gregor, P. D., Novina, C. D., Martinez, E., and Roeder, R. G. (1997) EMBO J. 16, 7091–7104
2. Roy, A. L., Malik, S., Meisterernst, M., and Roeder, R. G. (1991) Nature 354, 245–248
3. Roy, A. L., Malik, S., Meisterernst, M., and Roeder, R. G. (1993) Nature 365, 353–359
4. Cheriyath, V., and Roy, A. L. (2000) J. Biol. Chem. 275, 26300–26308
5. Cheriyath, V., and Roy, A. L. (2001) J. Biol. Chem. 276, 8377–8383
6. Roy, A. L. (2001) Gene (Amst.) 274, 1–13
7. Novina, C. D., Cheriyath, V., and Roy, A. L. (1998) J. Biol. Chem. 273, 33443–33448
8. Kim, D.-W., Cheriyath, V., Roy, A. L., and Cochran, B. H. (1998) Mol. Cell. Biol. 18, 3310–3320
9. Novina, C. D., Kumar, S., Bajpai, U., Cheriyath, V., Zhang, K., Pillai, S., Worts, H. H., and Roy, A. L. (1999) Mol. Cell. Biol. 19, 5014–5024
10. Kim, D.-W., and Cochran, B. H. (1998) Mol. Cell. Biol. 18, 4444–4451
11. Alberts, A. S., Geneste, O., and Treisman, R. (1998) J. Biol. Chem. 273, 33443–33448
12. Alberts, A. S., Geneste, O., and Treisman, R. (1998) Cell 92, 475–487
13. Alberts, A. S., Geneste, O., and Treisman, R. (1998) Mol. Cell. Biol. 18, 4444–4451
14. Gao, J., Zoller, K. E., Ginsberg, M. H., Brugge, J. S., and Shattil, S. J. (1997) EMBO J. 16, 6414–6425
15. Brown, M. T., and Cooper, J. A. (1996) Annu. Rev. Cell Dev. Biol. 12, 276, 274, 26300–26308
16. Thomas, S. M., and Brugge, J. S. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 604–609
17. Novina, C. D., Cheriyath, V., Novina, C. D., and Roy, A. L. (1998) Mol. Cell. Biol. 18, 4444–4451
18. Roy, A. L., Meisterernst, M., Pognonec, P., and Roeder, R. G. (1991) Nature 353, 245–248
19. Roy, A. L., Malik, S., Meisterernst, M., and Roeder, R. G. (1993) Nature 365, 353–359
20. Cheriyath, V., Novina, C. D., and Roy, A. L. (1998) Mol. Cell. Biol. 18, 4444–4451
21. Morris, M. C., Robert-Hebman, V., Chaolin, L., Mery, J., Heitz, F., Devaux, C., Goody, R. S., and Divita, G. (1999) J. Biol. Chem. 274, 24941–24946
22. Kim, D.-W., and Cochran, B. H. (2001) Mol. Cell. Biol. 21, 3387–3397
23. Parganas, E., Wang, D., Stravopodis, D., Topham, D. J., Marine, J., Teglund, S., Vanin, E. F., Bodner, S., Colamonici, O. R., and Ihle, J. N. (1998) Cell 93, 385–396
24. Abram, C. L., and Courtenidge, S. A. (2000) Exp. Cell Res. 254, 1–13
25. Brown, M. T., and Cooper, J. A. (1996) Biochim. Biophys. Acta 1287, 121–149
26. Thomas, S. M., and Brugge, J. S. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 604–609
27. Brugge, J. S., and Erikson, R. L. (1977) Nature 269, 346–348
28. Yamashita, A., and Treisman, R. (1998) J. Biol. Chem. 273, 33443–33448
29. Alberts, A. S., Geneste, O., and Treisman, R. (1998) Cell 92, 475–487
30. Alberts, A. S., Geneste, O., and Treisman, R. (1998) Mol. Cell. Biol. 18, 4444–4451
31. Novina, C. D., Cheriyath, V., and Roy, A. L. (1998) Mol. Cell. Biol. 18, 4444–4451
32. Valius, M., and Kazlauskas, A. (1993) Cell 73, 321–334
33. Hakak, Y., and Martin, G. S. (1999) Mol. Cell. Biol. 19, 6953–6962
34. Karmi, R., Jove, R., and Levitzki, A. (1999) Oncogene 18, 4654–4662
35. Yu, C. L., Meyer, D. J., Campbell, G. S., Larson, A. C., Carter-Su, C., Schwartz, J., and Jove, R. (1995) Science 269, 81–83
36. Wary, K. K., Mainiero, F., Isakoff, S. J., Marcantonio, E. E., and Giancotti, F. G. (1999) J. Biol. Chem. 274, 7321–7327
37. Hatakeyama, S., and Shiraishi, N. (1996) Cell 84, 693–702
38. Boyce, B. P., Yoneo, T., Lowe, C., Soriano, P., and Mundy, G. R. (1992) J. Clin. Invest. 90, 1622–1627
39. Tominga, T., Sahai, E., Chardin, P., McCormick, F., Courtenidge, S. A., and Alberts, A. S. (2006) Mol. Cell 5, 13–25
40. Perez-Jurado, L. A., Wang, Y.-K., Peoples, R., Coloma, A., Cruces, J., and Francke, U. (1998) Hum. Mol. Genet. 7, 325–334
41. Francke, U. (1999) Hum. Mol. Genet. 8, 1947–1954
