# The Simian Virus 40 Large Tumor Antigen Activates cSrc and Requires cSrc for Full Neoplastic Transformation

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**Abstract.** *Aim: To investigate the role of the cellular protooncogene product, cSrc, in neoplastic transformation by the large tumor antigen of simian virus 40 (TAg), the ability of TAg to increase cSrc activity was examined. Materials and Methods: cSrc activity was measured in cells expressing wild-type or mutant TAg and compared to the parental line. Results: The results indicated that TAg expression in mouse 3T3 fibroblasts causes a dramatic increase in cSrc activity, a finding which establishes TAg as a cSrc activator. This ability depended upon a TAg, intact retinoblastoma-susceptibility gene product (Rb) family-binding site. In addition, genetic ablation of pRb in mouse fibroblasts increased cSrc activity, suggesting that pRb inactivation by TAg might be responsible for the observed cSrc activation. Furthermore, down-regulation or genetic ablation of cSrc alone, or together with the Src family members, Yes and Fyn, caused a dramatic reduction in the ability of TAg to transform mouse fibroblasts. Conclusion: Taken together, these findings suggest for the first time that cSrc is part of an important pathway emanating from TAg and leading to neoplastic conversion.*

The simian virus 40 large tumor antigen (TAg) is a viral oncogene which is able to elicit neoplastic transformation in a variety of mammalian cell types by targeting a number of proteins to override cellular growth controls. Prominent among the latter are two tumor-suppressor proteins, p53 and the retinoblastoma-susceptibility gene product family (pRb, p107, p130, reviewed in (1)). The interaction with the Rb family is through an LXCXE motif (residues 103-107), and mutants where this motif is altered are defective for transformation in nearly all assay systems (2, 3).

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The effects of TAg on the Rb family proteins are thought to be exerted by regulating the activity of the E2F transcription factors. There are eight known E2F proteins (E2F1-8), all of which possess a DNA-binding domain that governs their interactions with a common consensus sequence present in the promoters of a number of genes (reviewed in (4, 5)). In quiescent cells, E2F-regulated genes are not expressed because their promoters are occupied primarily by p130/E2F4 complexes which repress transcription. Following receptor stimulation, Rb proteins are inactivated through phosphorylation by the cyclin-dependent kinases and this results in the replacement of the p130/E2F4 complexes by the 'activating' E2F1-3. This leads to the transcription of E2F-regulated genes, many of which encode proteins required for DNA replication, nucleotide metabolism, DNA repair and cell cycle progression. Large T antigen short-circuits this pathway by binding Rb proteins thereby blocking their ability to down-regulate E2Fs. Thus, TAg disrupts repressive Rb-E2F complexes, allowing transcription of E2F-dependent genes and progression of cells into the S-phase. In addition, the amino-terminus of TAg has a DNA-J domain function, which can recruit the heat-shock protein hsc70 to aid in the disruption of the Rb/E2F complex (reviewed in (6)).

Previous results demonstrated that TAg activates the Ras/Raf/extracellular signal regulated kinase (Erk) and signal transducer and activator of transcription-3 (Stat3) pathways (7-9), thus establishing a link between a viral oncogene known to have mainly nuclear targets, and the membrane signalling apparatus. However, the role of the cellular Src protooncogene product (cSrc), a signal transducer also often activated by membrane growth factor receptors, in TAg signalling is unclear (10). In the present communication, we attempted to examine the role of cSrc in transformation by TAg.

## Materials and Methods

*Cell lines, culture techniques and gene expression.* Tissue culture medium (Dulbecco's modified Eagle's medium, DMEM) was from ICN (Aurora, Ohio) and calf serum from Life Technologies Inc. (Burlington, ON, Canada). 3T3 fibroblasts were previously described (11) and were grown in plastic dishes in DMEM supplemented with 10% calf serum, in a 7% $CO_2$ incubator. The SYF, SYF-Src and Src++ cells (12) were obtained from ATCC

(Manassas, VA, USA) and grown in DMEM supplemented with 10% fetal calf serum.

TAg expression was achieved through a pBabe-Hygro-based retroviral vector system as described elsewhere (8). The K1 mutant, which is defective in pRb binding, was expressed with a pBabe-puro retroviral vector (a gift of Drs. Thomas Roberts and Ole Gjoerup). In each case, 3T3 fibroblasts were infected, selected for hygromycin resistance and a number of independent clones picked and tested for TAg levels. Representative clones were chosen for further study. The adenovirus vectors expressing the dominant-negative Src mutant (DN-Src) and the C-terminal Src kinase (Ad-Csk) which phosphorylates Src, Fyn and Yes kinases were a generous gift of Dr. D. R. Kaplan. They were amplified in 293 cells as elsewhere (9). Both vectors also express a green fluorescence protein (GFP) from a separate cytomegalovirus (CMV) promotor, which allows for easy identification of infected cells. High titer virus stocks were produced and the virus purified by CsCl centrifugation and titrated on 293 cells. Cells were infected with the vectors or the control pAdTrack (Stratagene, La Jolla, CA, USA) lacking an insert, at 300 pfu/ml in the presence of poly-lysine (13), and lysed 48 hours later for Western blotting, or for agar assays. In both cases, infection rates were more than 95%, as determined by fluorescence microscopy of the GFP protein (14). For growth rate assessment, cells were reinfected 4 days later.

To examine the cells' ability for anchorage-independent proliferation, approximately $10^4$ cells were suspended in 2 ml of 0.33% Agarose (Sigma, Oakville, ON, Canada)-containing DMEM supplemented with 15% fetal calf serum on top of a feeder layer of the same medium containing 0.7% agarose, in 6 cm petri dishes (8). Growth was recorded and photographs taken 10 days later under phase-contrast illumination. For foci formation, 200 3T3, 3T3-TAg, 3T3-TAg-DNSrc, SYF, SYF-Src or Src++ cells were plated together with $2\times10^4$ normal 3T3 cells. Foci appeared 10 days later and were stained and photographed.

*Western blotting*. Cells were grown to different densities from 10% to 2 days after confluence, at which times total proteins were extracted. In initial experiments, cells were lysed directly on the plate and protein determination conducted on extracts clarified by centrifugation. However, a significant amount of serum proteins present in the growth medium was found to attach nonspecifically to the plastic petri dish and be eluted with the detergent-containing extraction buffer, which could disturb the determination of protein concentration in the lysate significantly, especially at lower cell densities (15). To avoid this problem, cells were scraped in ice-cold phosphate-buffered saline (PBS), transferred into microcentrifuge tubes, washed once in PBS and the extraction buffer [50 mM Hepes, pH 7.4, 150 mM NaCl, 10 mM EDTA, 10 mM $Na_4P_2O_7$, 100 mM NaF, 2 mM $Na_3VO_4$, 0.5 mM phenylmethylsulphonyl fluoride (PMSF), 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1% Triton X-100 (16)] added to cell pellets for 10 minutes with vigorous pipetting. Subsequently, 30 μg of clarified cell extract were resolved on a 10% polyacrylamide-SDS gel and transferred to a nitrocellulose membrane (Bio-Rad, Mississauga, ON, Canada). Immunodetection was performed using antibodies against TAg (clone 108, a gift of Dr. Gurney), Src418 (#44-660G, Biosource, Carlsbad, CA, USA), total Src (36D10; Cell Signaling, Danvers, MA, USA, #2109), focal adhesion kinase (FAK) ptyr861 (#44-626G, Biosource) and α-tubulin (Cell Signalling), followed by alkaline phosphatase-conjugated goat secondary antibodies (#ALI 4405, Biosource,). The bands were visualized using enhanced chemiluminescence (ECL), according to the manufacturer's instructions (PerkinElmer Life Sciences, Waltham, MA, USA). Quantification was achieved by fluorimager analysis using the FluorChem program (AlphaInnotech Corp, San Leandro, CA, USA), with the values obtained normalized for α-tubulin.

## Results

*Cell density does not up-regulate cSrc activity in mouse 3T3 fibroblasts*. We previously demonstrated that cell confluence alone increases the activity of a number of proteins, such as the Jak kinases [(17), reviewed in (18)]. Therefore, to examine the role of Src in TAg-mediated transformation, we at first investigated the effect of cell density upon the levels of cSrc tyr418 phosphorylation (Src418) which correlates with its activity, in newly established mouse embryo 3T3 fibroblasts. As a control for protein loading, the same extracts were probed for α-tubulin (see Materials and Methods). As shown in Figure 1 (lanes 1-4), cell density had no effect upon Src418 or total Src protein levels, indicating that, unlike the Jak kinases (17), density does *not* affect cSrc activity.

*TAg triggers cSrc activation*. Previous results indicated that TAg can activate the Ras/Raf/Erk pathway, a finding which provided a link between a nuclear oncogene and the membrane signalling apparatus (7, 8). Since cSrc is also often activated by membrane-bound, growth factor receptors, we examined the ability of TAg to activate cSrc in mouse fibroblasts. To this effect, we used established mouse 3T3 fibroblasts expressing TAg with a retroviral vector (3T3-TAg), with cells infected with the same vector lacking an insert serving as negative controls [3T3 (8)]. As shown in Figure 1A, TAg expression caused a dramatic increase in Src418 levels, while total cSrc levels remained unchanged (lanes 1-4 *vs*. 5-8). To ensure that cell density did not affect TAg expression itself, TAg levels were also examined at different densities and found to be unchanged (Figure 1A, top panel). As a control for specificity of the TAg-mediated Src418 increase, Src418 levels were examined in 3T3 cells stably expressing activated $Ras^{leu61}$ (19) and found to have the same levels as the parental line (lanes 9-10), indicating that Src418 phosphorylation is not simply a general outcome of the transformed state. The above data taken together indicate that TAg expression results in stimulation of Src418 in cultured mouse fibroblasts.

One of the major Src substrates is FAK, which is phosphorylated by activated Src at tyr861 (20). To examine whether the increase in Src418 translates into an increase in Src activity, Western blots from TAg-expressing cells were probed with an antibody specific for FAK ptyr861 (21). The results (Figure 1A, right panel) revealed a dramatic increase in FAK ptyr861 upon TAg expression, indicating that TAg does indeed increase cSrc activity.

*TAg requires cSrc activity for neoplastic transformation*. To examine the cSrc requirement for TAg-induced transformation, cSrc activity was reduced in 3T3-TAg cells through infection

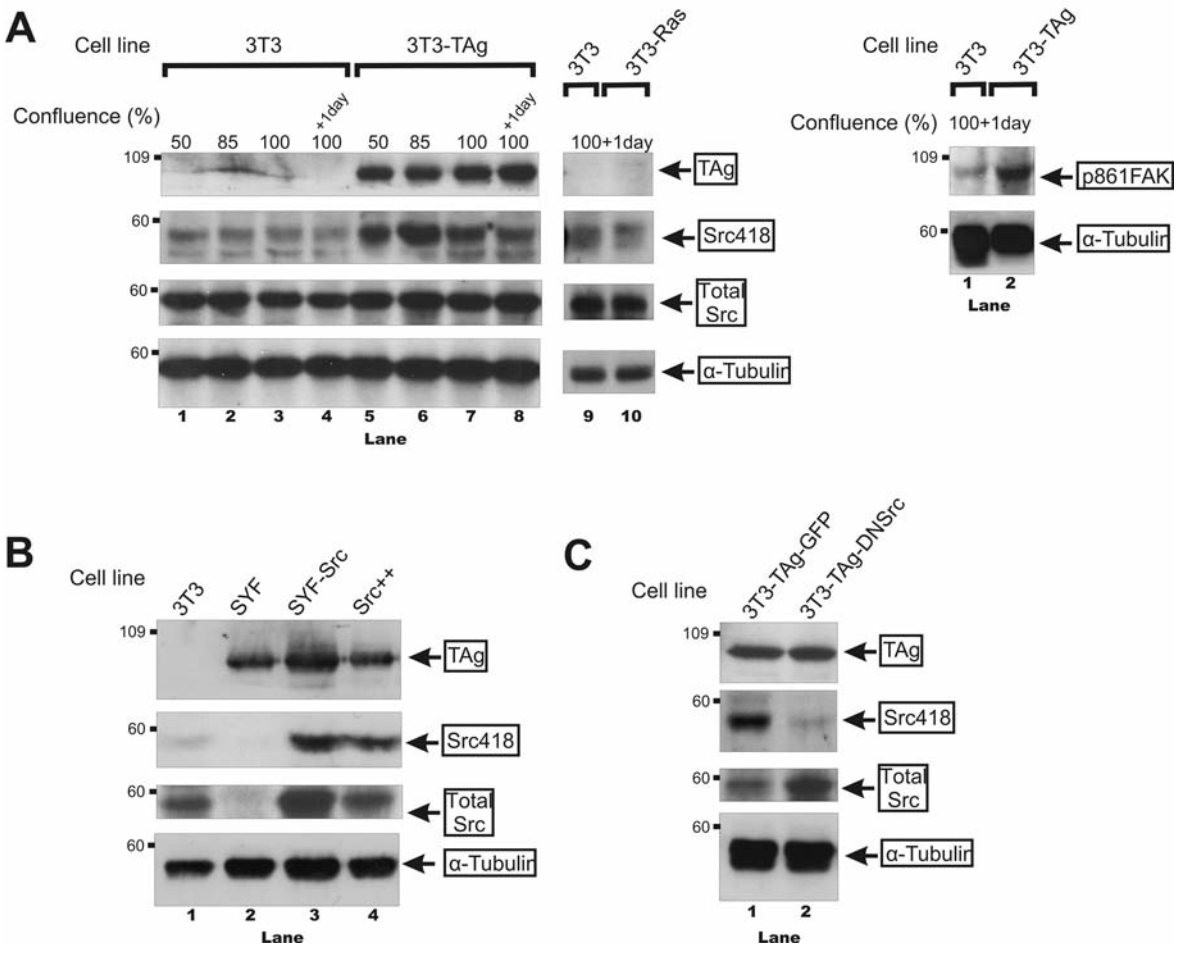


Figure 1. *TAg triggers cSrc activation. A, TAg activates cSrc in mouse 3T3 fibroblasts. Left panel: Control 3T3 fibroblasts infected with a blank vector (3T3, lanes 1-4) or their TAg-transformed counterparts (lanes 5-8) were grown to different densities as indicated and detergent lysates probed for TAg, Src418, total Src, and α-tubulin as a loading control, as indicated. As a negative control, detergent extracts from Ras-transformed 3T3 cells were probed for the same proteins (lanes 9-10). Numbers at the left refer to molecular weight markers. Right panel: Extracts from 3T3 or 3T3-TAg cells grown to one day post-confluence were probed for FAK p861, and α-tubulin as a loading control, as indicated. Numbers at the left refer to molecular weight markers. B, Src and TAg levels in SYF cells and their derivatives. Extracts from 3T3 fibroblasts (lane 1), cells where the Src, Fyn, Yes genes were ablated (SYF cells) before (lane 2) or after re-expression of Src (lane 3), or cells where Fyn and Yes only were ablated (Src++ cells, lane 4) were probed for TAg, Src418, total Src and α-tubulin as a loading control, as indicated. Numbers at the left refer to molecular weight markers. C, Src down-regulation following expression of DN-Src. 3T3-TAg cells were infected with an adenovirus vector containing DNSrc (lane 2), or the control vector expressing GFP alone (lane 1). Detergent cells lysates were were probed for TAg, Src418, and α-tubulin as a loading control, as indicated. Numbers at the left refer to molecular weight markers.*

with an adenovirus vector expressing a dominant-negative cSrc mutant (DNSrc, K297R, see Materials and Methods). As a control, cells were infected with the same vector expressing GFP alone. Western blotting 48 hours post infection indicated that Src418 levels were effectively reduced upon infection with this vector (Figure 1C). 3T3, 3T3-TAg and 3T3-TAg-DNSrc cells were subsequently tested for transformation-related parameters, such as morphological transformation, growth rate in monolayer culture and anchorage independence (see Materials and Methods). As shown in Figure 2A, 3T3-TAg cells were able to grow in agar and had a higher growth rate than the parental 3T3 cells. However, expression of the DNSrc mutant reduced the ability of 3T3-TAg cells to grow in agar, as well as their growth rate on plastic (Figure 2A). Similar results were obtained with cells infected with a vector expressing the cSrc kinase (CSK, not shown). The above findings taken together indicate that cSrc down-regulation reduces the ability of TAg to transform established mouse 3T3 fibroblasts.

To definitively demonstrate the requirement for Src, as well as the Src-related proteins, Fyn and Yes in TAg-mediated transformation, we made use of mouse fibroblasts where these genes had been genetically ablated (SYF cells). TAg had been previously expressed in these cells in order to establish them in culture from mouse E9.5 embryos. In addition, to examine

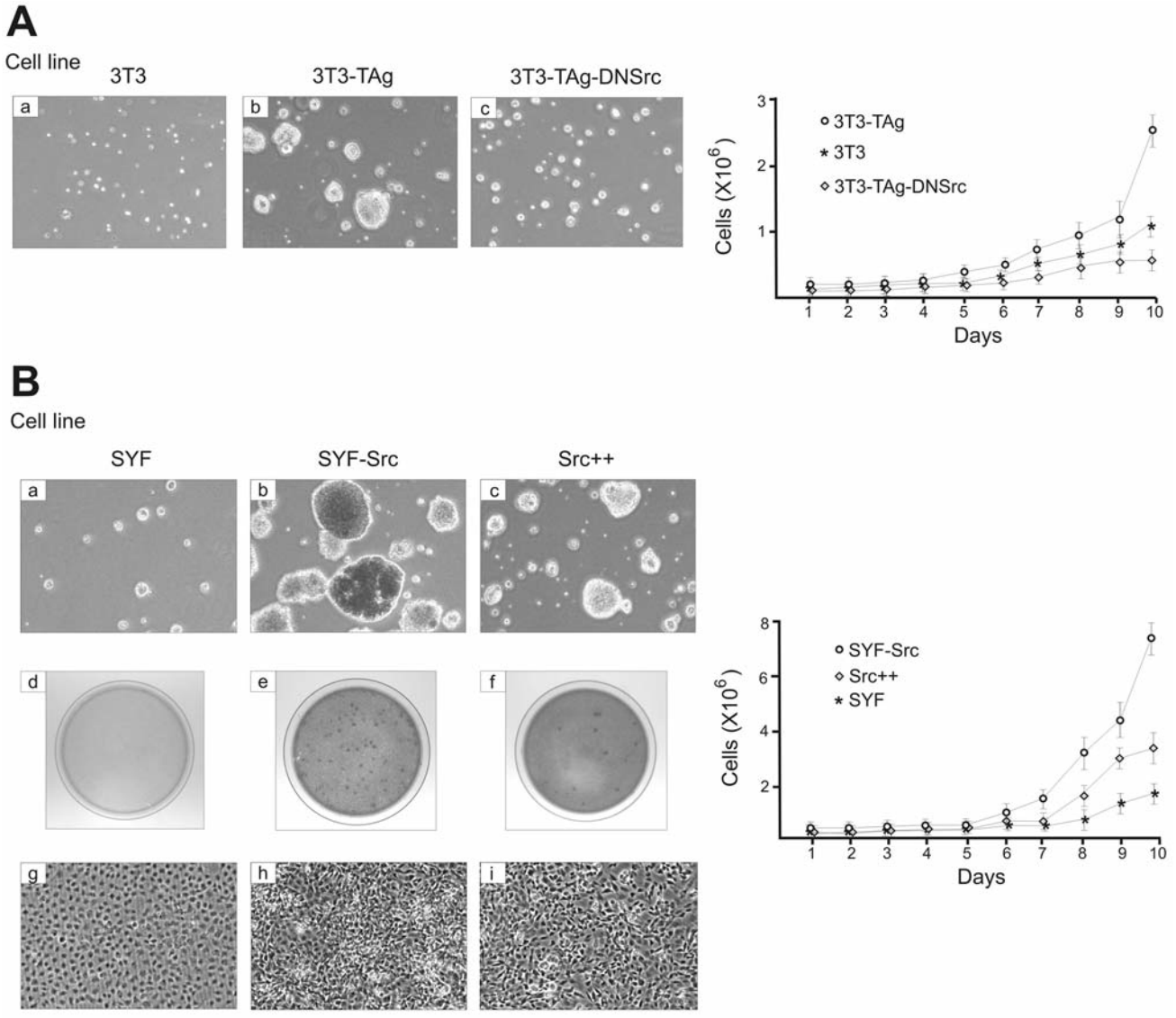


Figure 2. *Src deficiency prevents TAg-mediated transformation. A. Left panel: Anchorage-independent growth. (a) 3T3, (b) 3T3-TAg (c) 3T3-TAg-DNSrc cells were suspended in soft agarose. Twenty days later cells were photographed under phase contrast illumination. Magnification: ×40. Right panel: Growth rate on plastic. Src deficiency reduces the growth rate of 3T3-TAg cells. The indicated cell lines were plated in plastic, 6 cm petri dishes and cell numbers determined for several days, as indicated. B, Left panel: a-c: Agar assays. SYF, SYF-Src and Src++ cells were suspended in soft agarose. Twenty days later cells were photographed under phase-contrast illumination. Magnification: ×40. d-f: Formation of foci. Two hundred, SYF (a), SYF-Src (b), and Src++ (c) cells were plated in 6 cm petri dishes together with 10,000 normal 3T3 cells. Cells were fixed, stained with Coomassie blue and photographed 10 days later. g-i: Morphology on plastic. SYF, SYF-Src and Src++ cells were photographed under phase-contrast illumination. Magnification: ×140. Right panel: Growth rate on plastic. Src deficiency reduces the growth rate of 3T3-TAg cells. The indicated cell lines were plated in plastic, 6 cm petri dishes and cell numbers determined for several days, as indicated.*

the potential requirement for the Src family proteins, Fyn and Yes alone, we used established fibroblasts where the cSrc gene had been added back to SYF cells through retroviral vector infection (SYF-Src cells), and Src++ cells, where only Fyn and Yes were ablated (12). Western blotting indicated that all three lines expressed similar levels of TAg (Figure 1B, top panel). However, SYF-Src cells expressed slightly higher cSrc levels than Src++ or 3T3 cells due to the fact that cSrc was added back in these cells by retroviral vector infection (12). Examination of the cellular phenotype regarding transformation-associated properties indicated that while SYF-Src cells displayed a transformed morphology on plastic and were able to grow in agar and to form foci overgrowing a monolayer of normal cells, in a manner indistinguishable from

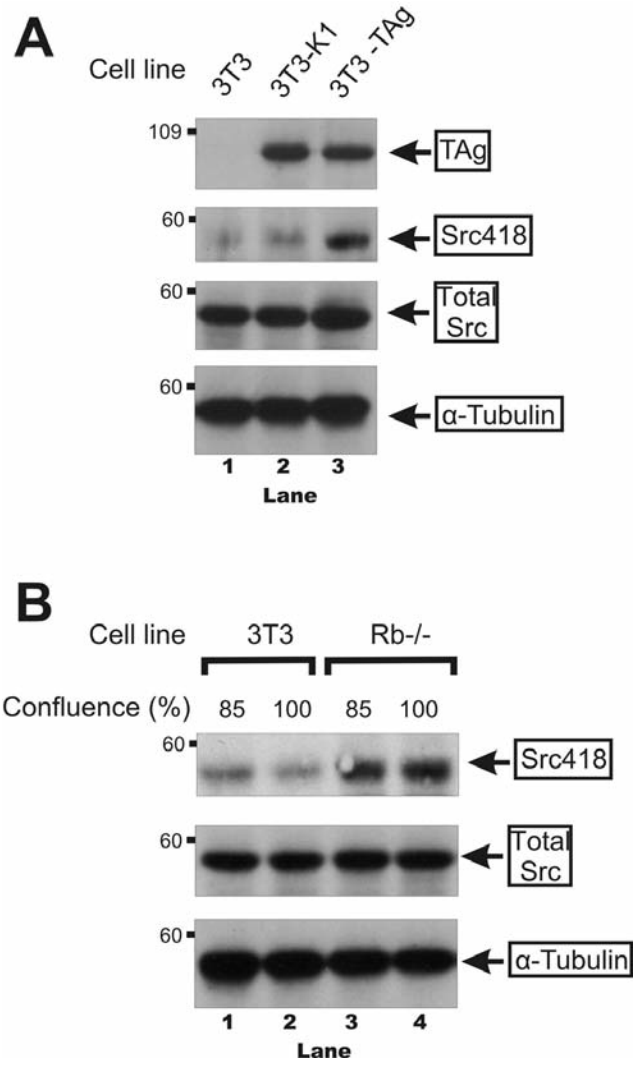


Figure 3. *pRb inactivation leads to cSrc activation. A, pRb binding by TAg is required for Src-tyr418 phosphorylation. Lysates from control 3T3 cells (lane 1) and 3T3 cells expressing the K1 TAg mutant which is defective in pRb binding (lane 2), or wt TAg (lane 3) were grown to different densities and Western immunoblots probed for TAg, Src418, total Src and or α-tubulin as a loading control, as indicated. Numbers at the left refer to molecular weight markers (see Materials and Methods). B, pRb ablation leads to cSrc, tyr418 phosphorylation. Lysates from 3T3 (lanes 1 and 2), and pRb-/- cells were probed for Src418, total Src and α-tubulin as a loading control, as indicated. Numbers at the left refer to molecular weight markers.*

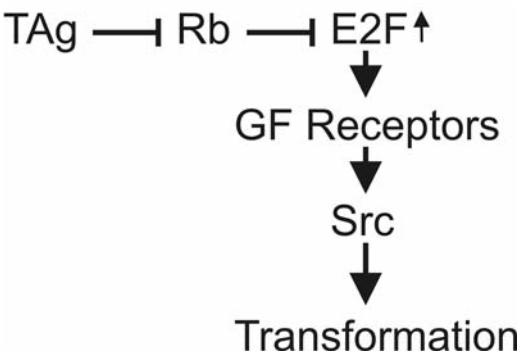


Figure 4. *Role of cSrc in transformation by TAg. TAg activates the transcription factor E2F through binding to and inactivation of pRb. E2F may then transcriptionally activate a number of receptor tyrosine kinases which could activate cSrc, leading to neoplastic conversion. GR, Growth Factor.*

3T3-TAg cells, the SYF cells had a slower growth rate, a flat morphology and were unable to grow as foci or in an anchorage-independent manner (Figure 2B), indicating that cSrc alone, in the absence of Fyn and Yes is sufficient to promote TAg-mediated transformation. The above results taken together indicate that cSrc is an integral component of the pathway whereby TAg transforms mouse fibroblasts. *pRb binding is required for cSrc activation by TAg*. One of the TAg targets is the retinoblastoma-susceptibility gene product (pRb). To investigate the importance of pRb binding to and inactivation by TAg, for TAg-induced cSrc activation and neoplasia, we examined the ability of the non-transforming TAg mutant K1 (E107K), which is unable to bind pRb family proteins, to increase Src activity levels. As shown in Figure 3A, Src activity levels in K1-expressing 3T3 cells were low, similar to that of the parental 3T3 line (lanes 1 and 2, respectively), indicating that Src activation is associated with the ability of TAg to bind pRb and neoplastically transform rodent fibroblasts.

To definitively demonstrate the role of pRb inactivation in Src activation by TAg, we measured Src418 levels in cells established from knockout mice where the pRb gene had been genetically ablated ($pRb^{-/-}$ cells) (11). pRb deletion in these cells liberates the activating E2F transcription factors and abrogates the $G_1$ restriction point. As shown in Figure 3B, the $pRb^{-/-}$ cells had high Src418 levels compared to their wild-type counterparts (lanes 3-4, *vs*. 1-2). The above data taken together point to the possibility that inactivation of pRb by TAg may induce the observed Src 418 phosphorylation and activation, leading to neoplastic conversion.

## Discussion

We examined the role of the cellular Src protooncogene product in transformation by TAg. The results demonstrated that TAg expression causes a dramatic increase in Src418 phosphorylation and activity, measured by FAK tyr861 phosphorylation, in the absence of an increase in total cSrc protein. In addition, down-regulation of cSrc activity through expression of a dominant-negative mutant, expression of the cSrc kinase or genetic ablation reduced the cells' growth rate, focus formation ability and anchorage-independence, pointing to a requirement for Src

function in TAg-mediated transformation. Thus, albeit predominantly nuclear and thought to affect mostly nuclear targets, TAg requires the activity of the membrane-bound cSrc to induce full neoplastic conversion.

We and others previously demonstrated that cell to cell adhesion dramatically increases the levels and activity of a number of proteins, such as Rac (22), connexin-43 (23) as well as Stat3 [reviewed in (18)]. Since Src is an important Stat3 activator, we examined the effect of cell density upon Src tyr418 phosphorylation, previously shown to correlate with activity. Our results demonstrated the absence of an increase in Src 418 phosphorylation with cell density. This finding is consistent with previous results demonstrating that the cell density-dependent Stat3 activation is independent of Src action (17). In fact, cadherin engagement, as brought about through confluence of cultured cells triggers a dramatic increase in the levels of interleukin-6 (IL6), which leads to Stat3 ptyr705 phosphorylation and activation through the Jak kinases, rather than Src (22). Consistent with this observation, IL-6 addition did not increase Src418 levels in any of our cellular systems (not shown), as previously documented by others (24).

The cSrc protein is composed of a *C*-terminal tail containing a negative regulatory tyrosine residue (tyr529), which interacts with the cSrc SH2 domain. In addition, the SH3 domain interacts with the kinase domain and this causes the cSrc molecule to assume a closed configuration that covers the kinase domain and reduces the potential for substrate interaction (25). The tyr529 phosphorylation is conducted by CSK kinase (26). Conversely, the phosphate residue on tyr529 can be removed by several phosphatases that are potent cSrc activators. One of them is the protein tyrosine phosphatase 1b (PTP1b), the first phosphatase to be identified and cloned (27). Upon overexpression in breast cancer lines, PTP1b was shown to depho-sphosphorylate Src ptyr529 and activate cSrc (28). However, using PTP1b knockout lines, rather than overexpression experiments, it was later shown that PTP1b can dephosphorylate and activate cSrc in mouse fibroblasts, but only when the cells are kept in suspension (29). Since however, TAg expression leads to a dramatic increase in PTP1b levels (29), the possibility remains that TAg-mediated PTP1b activation might be a significant contributor to cSrc activation.

The pRb family of anti-oncogenes are important TAg targets (1), and the TAg–Rb interaction is required for transformation, even by cytoplasmic TAg mutants (30). Current models of pRb function indicate that the TAg–pRb association, in turn, inhibits pRb binding to the E2F family of transcription factors, which are important cell cycle regulators (5). In fact, a detailed examination of E2F-activated genes indicated that E2F1 has many targets, among which is a number of membrane receptor tyrosine kinases and their ligands, including known Src activators (31), such as epidermal growth factor receptor (EGFR) (32), platelet-derived growth factor α receptor (PDGFαR) (33, 34), fibroblast growth factor receptor (35), colony-stimulating factor 1 (36), and hepatocyte growth factor (37). These can induce cSrc activity, probably by disrupting the intramolecular interactions that hold cSrc in a closed configuration. Such an induction of growth factor or receptor genes by E2F following TAg expression would explain the observed cSrc activation. The fact that, like TAg levels, cSrc activity does *not* increase with cell density in TAg-expressing cells (Figure 1A, lanes 5-8) is consistent with this observation. Furthermore, the fact that the pRb-binding site is required both for transformation by TAg and for cSrc activation highlights the importance of cSrc in TAg action (Figure 4). This conclusion is further reinforced by the fact that pRb inactivation by genetic ablation also leads to cSrc activation. Thus, it appears that pRb inactivation is an important factor both for TAg-mediated cSrc activation and neoplastic transformation. The present report presents evidence for cSrc as an integral component of the signaling pathways from the primarily nuclear TAg oncogene.

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## References

1 Ahuja D, Saenz-Robles MT and Pipas JM: SV40 large T antigen targets multiple cellular pathways to elicit cellular transformation. Oncogene *24*: 7729-7745, 2005.

2 Chen S and Paucha E: Identification of a region of simian virus 40 large T antigen required for cell transformation. J Virol *64*: 3350-3357, 1990.

3 Zalvide J and DeCaprio JA: Role of pRb-related proteins in simian virus 40 large-T-antigen- mediated transformation. Mol Cell Biol *15*: 5800-5810, 1995.

4 Attwooll C, Lazzerini DE and Helin K: The E2F family: specific functions and overlapping interests. EMBO J *23*: 4709-4716, 2004.

5 Dimova DK and Dyson NJ: The E2F transcriptional network: old acquaintances with new faces. Oncogene *24*: 2810-2826, 2005.

6 Sullivan CS and Pipas JM: T antigens of simian virus 40: molecular chaperones for viral replication and tumorigenesis. Microbiol Mol Biol Rev *66*: 179-202, 2002.
7 Grammatikakis N, Jaronczyk K, Grammatikakis A, Vultur A, Brownell HL, Benzaquen M, Rausch C, Lapointe R, Gjoerup O, Roberts TM and Raptis L: Simian virus 40 large tumor antigen modulates the Raf signaling pathway. J Biol Chem *276*: 27840-27845, 2001.
8 Raptis L, Brownell HL, Wood K, Corbley M, Wang D and Haliotis T: Cellular ras gene activity is required for full neoplastic transformation by simian virus 40. Cell Growth Differ *8*: 891-901, 1997.
9 Vultur A, Arulanandam R, Turkson J, Niu G, Jove R and Raptis L: Stat3 is required for full neoplastic transformation by the simian virus 40 large tumor antigen. Mol Biol Cell *16*: 3832-3846, 2005.
10 Broome MA and Courtneidge SA: No requirement for src family kinases for PDGF signaling in fibroblasts expressing SV40 large T antigen. Oncogene *19*: 2867-2869, 2000.
11 Sage J, Mulligan GJ, Attardi LD, Miller A, Chen S, Williams B, Theodorou E and Jacks T: Targeted disruption of the three Rb-related genes leads to loss of G(1) control and immortalization. Genes Dev *14*: 3037-3050, 2000.
12 Klinghoffer RA, Sachsenmaier C, Cooper JA and Soriano P: Src family kinases are required for integrin but not PDGFR signal transduction. EMBO J *18*: 2459-2471, 1999.
13 Orlicky DJ and Schaack J: Adenovirus transduction of 3T3-L1 cells. J Lipid Res *42*: 460-466, 2001.
14 Angers-Loustau A, Hering R, Werbowetski TE, Kaplan DR and Del Maestro RF: SRC regulates actin dynamics and invasion of malignant glial cells in three dimensions. Mol Cancer Res *2*: 595-605, 2004.
15 Greer S, Honeywell R, Geletu M, Arulanandam R and Raptis L: housekeeping gene products; levels may change with confluence of cultured cells. Journal of Immunological Methods JIM-D-09-00266: 2009.
16 Raptis L, Brownell HL, Vultur AM, Ross G, Tremblay E and Elliott BE: Specific inhibition of growth factor-stimulated ERK1/2 activation in intact cells by electroporation of a Grb2-SH2 binding peptide. Cell Growth Differ *11*: 293-303, 2000.
17 Vultur A, Cao J, Arulanandam R, Turkson J, Jove R, Greer P, Craig A, Elliott BE and Raptis L: Cell to cell adhesion modulates Stat3 activity in normal and breast carcinoma cells. Oncogene *23*: 2600-2616, 2004.
18 Raptis L, Arulanandam R, Vultur A, Geletu M, Chevalier S and Feracci H: Beyond structure, to survival: Stat3 activation by cadherin engagement. Biochem Cell Biol *87*: 835-851, 2009.
19 Raptis L, Yang J, Brownell HL, Lai J, Preston T, Corbley MJ, Narsimhan RP and Haliotis T: Rasleu61 blocks differentiation of transformable 3T3 L1 and C3HT1/2-derived preadipocytes in a dose- and time-dependent manner. Cell Growth Differ *8*: 11-21, 1997.
20 Calalb MB, Zhang X, Polte TR and Hanks SK: Focal adhesion kinase tyrosine-861 is a major site of phosphorylation by Src. Biochem Biophys Res Commun *228*: 662-668, 1996.
21 Gabarra-Niecko V, Schaller MD and Dunty JM: FAK regulates biological processes important for the pathogenesis of cancer. Cancer Metastasis Rev *22*: 359-374, 2003.
22 Arulanandam R, Vultur A, Cao J, Carefoot E, Truesdell P, Elliott B, Larue L, Feracci H and Raptis L: Cadherin engagement promotes survival *via* Rac/Cdc42 and Stat3. Mol Cancer Res *7*: 1310-1327, 2009.
23 Geletu M, Chaize C, Arulanandam R, Vultur A, Kowolik C, Anagnostopoulou A, Jove R and Raptis L: Stat3 activity is required for gap junctional permeability in normal epithelial cells and fibroblasts. DNA Cell Biol *28*: 319-327, 2009.
24 Fischer P and Hilfiker-Kleiner D: Role of gp130-mediated signalling pathways in the heart and its impact on potential therapeutic aspects. Br J Pharmacol *153(Suppl 1)*: S414-S427, 2008.
25 Yeatman TJ: A renaissance for SRC. Nat Rev Cancer *4*: 470-480, 2004.
26 Cooper JA, Gould KL, Cartwright CA and Hunter T: Tyr 527 is phosphorylated in pp60c-src: Implications for regulation. Science *231*: 1431-1434, 1986.
27 Chernoff J, Schievella AR, Jost CA, Erikson RL and Neel BG: Cloning of a cDNA for a major human protein-tyrosine phosphatase. Proc Natl Acad Sci USA *87*: 2735-2739, 1990.
28 Bjorge JD, Pang A and Fujita DJ: Identification of protein-tyrosine phosphatase 1B as the major tyrosine phosphatase activity capable of dephosphorylating and activating c-Src in several human breast cancer cell lines. J Biol Chem *275*: 41439-41446, 2000.
29 Cheng A, Bal GS, Kennedy BP and Tremblay ML: Attenuation of adhesion-dependent signaling and cell spreading in transformed fibroblasts lacking protein tyrosine phosphatase-1B. J Biol Chem *276*: 25848-25855, 2001.
30 Tedesco D, Fischer-Fantuzzi L and Vesco C: Limits of transforming competence of SV40 nuclear and cytoplasmic large T mutants with altered Rb binding sequences. Oncogene *8*: 549-557, 1993.
31 Young AP, Nagarajan R and Longmore GD: Mechanisms of transcriptional regulation by Rb-E2F segregate by biological pathway. Oncogene *22*: 7209-7217, 2003.
32 Tice DA, Biscardi JS, Nickles AL and Parsons SJ: Mechanism of biological synergy between cellular Src and epidermal growth factor receptor. Proc Natl Acad Sci USA *96*: 1415-1420, 1999.
33 DeMali KA, Godwin SL, Soltoff SP and Kazlauskas A: Multiple roles for Src in a PDGF-stimulated cell. Exp Cell Res *253*: 271-279, 1999.
34 Bowman T, Broome MA, Sinibaldi D, Wharton W, Pledger WJ, Sedivy JM, Irby R, Yeatman T, Courtneidge SA and Jove R: Stat3-mediated Myc expression is required for Src transformation and PDGF-induced mitogenesis. Proc Nat Acad Sci USA *98*: 7319-7324, 2001.
35 Landgren E, Blume-Jensen P, Courtneidge SA and Claesson-Welsh L: Fibroblast growth factor receptor-1 regulation of Src family kinases. Oncogene *10*: 2027-2035, 1995.
36 Courtneidge SA, Dhand R, Pilat D, Twamley GM, Waterfield MD and Roussel MF: Activation of Src family kinases by colony stimulating factor-1, and their association with its receptor. EMBO J *12*: 943-950, 1993.
37 Mao W, Irby R, Coppola D, Fu L, Wloch M, Turner J, Yu H, Garcia R, Jove R and Yeatman TJ: Activation of c-Src by receptor tyrosine kinases in human colon cancer cells with high metastatic potential. Oncogene *15*: 3083-3090, 1997.

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