Rac1 Function Is Required for Src-induced Transformation

EVIDENCE OF A ROLE FOR TIA1 AND VAV2 IN RAC ACTIVATION BY SRC

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The proto-oncogene c-Src has been implicated in the development and progression of a number of human cancers including those of colon and breast. Accumulating evidence indicates that activated alleles of Src may induce cell transformation through Ras-ERK-dependent and -independent pathways. Here we show that Rac1 activity is strongly elevated in Src-transformed cells and that this small G protein is a critical component of the pathway connecting oncogenic Src with cell transformation. We further show that Vav2 and the ubiquitously expressed Rac1 guanine nucleotide exchange factor Tiam1 are phosphorylated in tyrosine residues in cells transfected with active and oncogenic Src. Moreover, phosphorylation of Tiam1 in cells treated with pervanadate, a potent inhibitor of tyrosine phosphatases, was partially inhibited by the Src inhibitor SU6656. Using truncated mutants of Tiam1, we demonstrate that multiple sites in Rac1 can be tyrosine-phosphorylated by Src. Furthermore, Tiam1 cooperated with Src to induce activation of Rac1 in vivo and the formation of membrane ruffles. Similarly, activation of JNK and the c-Jun promoter by Src were also potently increased by Tiam1. Together, these results suggest that Vav2 and Tiam1 may act as downstream effectors of Src, thereby regulating Rac1-dependent pathways that participate in Src-induced cell transformation.

The cytoplasmic tyrosine kinase Src was initially identified as the protein product of the first discovered viral oncogene, v-src, which is responsible for the transforming ability of the Rous sarcoma virus (1–3). Its normal cellular counterpart, the product of the proto-oncogene c-src (4, 5), was later found to play a key role in signal transduction processes by acting downstream from a large variety of cell surface receptors (reviewed in Ref. 6). c-Src was also found to be overexpressed and highly active in a number of distinct tumor types, including those affecting the colon, breast, pancreas, and central nervous system, where it may play an important role in tumor development and in the acquisition of the metastatic phenotype (reviewed in Ref. 7).

The molecular mechanisms underlying the transforming potential of v-Src have been extensively explored. In particular, expression of oncogenic and activated forms of Src results in tyrosine phosphorylation of numerous intracellular substrates and the consequent activation of multiple signaling pathways that ultimately control cell proliferation. For example, active Src can potently stimulate the Ras-ERK1 pathway, primarily by the tyrosine phosphorylation of Shc by Src, followed by the recruitment to the membrane of the adapter protein Grb2 and the Ras-guanine nucleotide exchange factor Sos, thereby promoting Ras activation (8). However, several studies have indicated that Src can also utilize biochemical routes distinct from the Ras-ERK pathway to promote its biological responses (9–11). Alternative pathways activated by Src may contribute to the activation of NF-xB, STAT3, and E2F that can then stimulate the expression of several genes implicated in cell cycle control, such as cyclin D and c-Myc (12–16). Of interest, the latter has been shown to be dependent on the activation of a GTP-binding protein of the Rho family, Rac1, but not on the Ras-ERK pathway (10).

Rho GTPases, including RhoA, Rac1, and Cdc42, can control transcriptional events in the nucleus in addition to their effects on the organization of the cellular cytoskeleton (17–19). In particular, Rac1 has emerged as a key upstream activator of multiple signaling pathways regulating gene expression. For example, Rac1 regulates the transcriptional activity of c-Jun through the JNK pathway (19, 20) and the serum-responsive element through the transcriptional activation of the serum response factor (17). As for other small GTPases, the functional activity of Rac1 is tightly regulated in vivo. Guanine nucleotide exchange factors (GEFs) activate these GTPases by promoting the exchange of their inactive GDP-bound forms to their active GTP-bound species. Most known GEFs for these proteins share a highly related structural domain of about 250 amino acids, termed the Dbl homology (DH) domain, which is adjacent to a pleckstrin homology (PH) domain (reviewed in Ref. 21). The biochemical connection between receptor and nonreceptor tyrosine kinases and the activation of Rac1 involves the activation of Rac GEFs and/or the inhibition of GTPase-activating proteins acting on Rac1. For example, we have shown that the tyrosine phosphorylation of the Rac1 exchange factor Vav1,
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which is predominantly expressed in hematopoietic cells, leads to the activation of the GDP/GTP exchange activity of Rac1 (22, 23). Interestingly, the exchange activity of Vav2, a more widely expressed member of the Vav family, is also increased upon tyrosine phosphorylation (24), suggesting that this GEF may be involved in Rac1 activation by Src kinases in nonhematopoietic cells. However, whether Rac1 activation plays a role in cell transformation by activated alleles of Src and, if so, the precise nature of the Rac GEFs involved are both still poorly defined.

In this study, we first examined the role of Rac1 activation in Src-induced transformation. We found that v-Src-transformed NIH 3T3 cells display remarkably high levels of GTP-bound Rac1 and that molecules interfering with the activation of Rac1 or the ability of Rac1 to induce downstream pathways potently inhibited the focus forming activity of v-Src. In search for the underlying mechanism leading to Rac1 activation, we found that endogenous Vav2, which is limitedly expressed in NIH 3T3 cells, is tyrosine-phosphorylated in v-Src transformed cells. In addition, we observed that Tiam1, a specific Rac1-GEF that is highly expressed in NIH 3T3 cells, is also tyrosine-phosphorylated in v-Src-transformed cells. Furthermore, we found that active Src strongly potentiates Tiam1-induced Rac1 activation and the consequent stimulation of cellular responses controlled by this small GTPase. These findings suggest that Tiam1 is a novel downstream effector of Src and that Src may utilize Tiam1 and Vav2 to regulate the activity of Rac1, thereby initiating the activation of a variety of cytoplasmic and nuclear events that are necessary for cellular transformation.

MATERIALS AND METHODS

Cell Lines and Transfections—NIH 3T3 fibroblasts were maintained in Dulbecco’s modified Eagle’s medium (Sigma-supplemented with 10% (v/v) calf serum (Bio Whittaker). To establish NIH 3T3 cells stably expressing v-Src (NIHv-Src), the cells were transfected by the calcium-phosphate precipitation technique with pCEFL-v-Src. The cells were selected in culture medium containing G418 (750 μg/ml) for 3 weeks. Human embryonic kidney (HEK) 293T cells were cultured in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum. Transient transfections in HEK 293T cells were performed using the LipofectAMINE Plus reagent (Invitrogen) according to the manufacturer’s instructions.

DNA Constructs.—The expression vectors pSM-SrcYF (constitutively active) and pSM-SrcYF-KM (dominant negative) were kindly provided by H. Varmus. The plasmids pCEFL-SrcYF and pCEFL-SrcYF-KM were generated by cloning SrcYF and SrcYF-KM in the mammalian expression vector pCEFL. The coding sequence for the Cdc42/Rac1 active) and pSM-SrcYF-KM (dominant negative) were kindly provided by J. Collard. The expression vector (pcDNAIII derivatives) were kindly provided by S. Momand. The expression vector pCEFL-EGFP was generated by cloning EGFP wild type in frame with the C-terminal EGFP coding sequence. Constitutively active forms of Rac1 were cloned into pCEFL in frame with the C-terminal EGFP coding sequence (24). Dominant negative forms of Rac1 (Rac1 N17) and Cdc42 (Cdc42 N17) were obtained by replacing threonine 17 for asparagine in their corresponding coding sequence. Reagents

Regulation of Protein Tyrosine Phosphorylation—For prediction of potential sites for tyrosine phosphorylation by members of the Src family, amino acid sequences were analyzed using the ScanSite (scan-site.mit.edu) and the NetPhos 2.0 prediction server (www.cbs.dtu.dk/services/NetPhos). For experimental determination of tyrosine phosphorylation, the proteins were immunoprecipitated under specific conditions, and the immunoprecipitates were subjected to Western blot analysis against phosphotyrosines using a combination of monoclonal antibodies from Upstate Biotechnology, Inc. (4G10) and Santa Cruz (PY-99). The proteins were visualized by enhanced chemiluminescence detection (Amersham Biosciences) using goat anti-mouse IgGs coupled to horseradish peroxidase as the secondary antibody (ICN). Staining

Firefly luciferase gene driven by a wild type murine c-jun promoter, pLuc, had been previously described (18). Double positive cells were fixed with 1× PBS solution containing 2% (v/v) formaldehyde and 0.2% (v/v) glutaraldehyde and stained at 37 °C for β-galactosidase activity with a 1× PBS solution containing 2 mM MgCl2, 5 mM K2Fe(CN)6, 5 mM K4Fe(CN)6, and 0.1% 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) to evaluate the transfection efficiency.

JNK Kinase Assay—HEK 293T cells were seeded at 70–80% confluence and transfected with Polyfect (Qiagen) with the expression vector HA-tagged JNK1 alone or in combination with other plasmids. After transfection, the cells were cultured for 18 h and incubated in serum-free medium for 2 h. The cells were washed with cold PBS and lysed at 4 °C in JNK lysis buffer containing 25 mM HEPES, pH 7.5, 0.5 mM NaCl, 1.5 mM MgCl2, 0.2 mM EDTA, 0.5 mM dithiothreitol, 20 μM β-glycerophosphate, 1 mM vanadate, 1× Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 20 μg/ml aprotinin, and 20 μg/ml leupeptin. HA-tagged JNK1 was immunoprecipitated from cleared lysates at 4 °C for 2 h with anti-HA monoclonal antibody (Babco). Immunocomplexes were recovered by addition of 1 μg of pDNAIH-β-gal, a plasmid expressing the enzyme β-galactosidase, adjusting the total amount of plasmid DNA with empty vector. The day after transfection, the cells were washed in medium supplemented with 5% calf serum and then maintained in the same medium for 2–3 weeks. The foci were stained and scored as described (18). Duplicate plates were fixed with 1× PBS solution containing 2% (v/v) formaldehyde and 0.2% (v/v) glutaraldehyde and stained at 37 °C for β-galactosidase activity with a 1× PBS solution containing 2 mM MgCl2, 5 mM K2Fe(CN)6, 5 mM K4Fe(CN)6, and 0.1% 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal) to evaluate the transfection efficiency.

Fluorescence Microscopy—Porcine aortic endothelial (PAE) cells were grown in 24-well plates on coverslips and transfected using FUGENE 6 (Roche Applied Science) with the pCEFL-EGFP plasmid along with additional expression vectors. The Cells were serum-starved for 8 h and washed twice with PBS. The cells were then fixed with 2% paraformaldehyde (in 1× PBS) for 20 min and permeabilized with 0.5%...
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Fig. 1. Rac1 is activated in v-Src-transformed NIH 3T3 cells. A, NIH 3T3 cells and NIH 3T3 cells stably transformed with v-Src (NIH/v-Src) were incubated in serum-free medium with vehicle (Me2SO) or SU6656 (2 μM) for 3 h. The lysates were subjected to Western blot (WB) analysis with anti-phosphotyrosine (PY) antibody. B, NIH 3T3 and NIH/v-Src cells were serum-deprived for 3 h, and the cell lysates were incubated with GST-PAK-N for 30 min to affinity precipitate (AP) active Rac1. PAK-bound Rac1 and total Rac1 in the corresponding total lysates (TL) were analyzed by WB with a monoclonal antibody against Rac1. C, NIH 3T3 cells were incubated with serum-free medium for 3 h and treated with PDGF for the indicated times. The levels of active Rac1 and total Rac1 were analyzed as in B.

Triton X-100 (in 1× PBS) for 10 min. The coverslips were blocked with 0.5% bovine serum albumin and incubated for 1 h with phalloidin conjugated to Texas Red. The digital images were captured using Spotcam.

RESULTS

Rac1 Is Constitutively Active in v-Src Transformed Cells—To examine the contribution of Rac1 in v-Src-mediated cell transformation, we first explored the status of Rac1 activity in v-Src-transformed cells. To this end, we established NIH 3T3 lines stably expressing v-Src (NIH/v-Src). As a control, these cells exhibited a substantially increased level of phosphotyrosine containing proteins, whose extent of phosphorylation was strongly reduced by the highly selective Src inhibitor SU6656 (28) (Fig. 1A). For the analysis of Rac1 activity in vivo, we used a GST fusion protein containing the CRIB domain of the PAK1, prebound to glutathione-Sepharose beads, to bind and affinity precipitate the activated, GTP-bound forms of Rac1. Using this assay, we observed remarkably high levels of Rac1-GTP in NIH/v-Src cells when compared with those in parental, nontransformed NIH 3T3 cells (Fig. 1B). Total Rac1 expression levels were comparable between these two cell populations, and although normal NIH 3T3 cells exhibited low levels of active Rac1 under basal conditions, the amount of active Rac1 in these cells was rapidly and transiently increased upon stimulation with PDGF, which served as a control (Fig. 1C). Thus, stable expression of v-Src results in a remarkable and persistent increase in the levels of active Rac1, comparable with that achievable by acute exposure of NIH 3T3 cells to polypeptide growth factors.

Transformation Induced by v-Src Is Inhibited by the Blockade of the Rac1 Pathway—We next explored the contribution of Rac1 to the ability of v-Src to transform NIH 3T3 cells. In this regard, we noticed that v-Src-induced foci are morphologically distinct from the spread out foci induced by an activated form of an upstream kinase of ERK1/2, MEK1 EE, which are typical of other oncopgenes stimulating the ERK pathway, such as ras and raf (29, 30) (Fig. 2). Indeed, v-Src-induced foci are highly compact and well delineated, resembling more closely the foci induced by members of the Rho GEF family and activated forms of Rho GTPases (30). When v-Src was cotransfected with expression vectors for either a dominant negative form of Rac1 (Rac1 N17), which interferes with Rac1 activation (31), or a GST fusion protein containing the CRIB domain of PAK (PAK-N), that prevents Rac1 function (32), the number of foci induced by v-Src were significantly reduced. In contrast, the number of foci induced by MEK1 EE was not affected by these Rac1-interfering constructs. Together, these results suggest that Rac1 is an integral component of the transforming pathway utilized by v-Src.

Vav2 and Tiam1 Are Phosphorylated on Tyrosine Residues by Active Src and Endogenous Tyrosine Kinases—On the basis of the above observations, we next set out to further define the mechanism underlying Rac1 activation by Src. Because the ubiquitously expressed Vav2 can be regulated by tyrosine kinases (24), we confirmed that endogenous Vav2 was tyrosine-phosphorylated in NIH/v-Src cells (Fig. 3A). However, the expression levels of this GEF for Rho GTPases are relatively low, and large amounts of cellular proteins need to be immunoprecipitated to detect Vav2 tyrosine phosphorylation. In addition, we have recently observed that dominant negative forms of Vav2 inhibit only partially the effects mediated by Src (10).
Thus, other Rac1 GEFs may also contribute to the activation of Rac1 by Src in addition to Vav2.

Among the several members of the growing superfamily of GEFs for Rho proteins, Tiam1 has been shown to have high specificity for Rac1 and to be widely expressed. Of interest, a detailed examination of the amino acid sequence of Tiam1 revealed the existence of potential consensus sites for phosphorylation by members of the Src family (scansite.mit.edu). Thus, we set out to determine whether Tiam1 was a Src effector leading to Rac1 activation. As a first step, we asked whether Src could induce Tiam1 phosphorylation on tyrosine residues. As shown in Fig. 3A, endogenous Tiam1 was clearly tyrosine-phosphorylated in NIH/v-Src cells, as judged by its immunodetection by anti-phosphotyrosine-specific antibodies in Tiam1 immunoprecipitates.

We next investigated whether an HA-tagged Tiam1 can be phosphorylated in cells transiently transfected with an activated form of Src. As shown in Fig. 3B, immunoprecipitated Tiam1 was only slightly phosphorylated by overexpression of c-Src, whereas an active Src (SrcYF), in which Tyr527 was mutated to Phe, induced a much stronger effect. Both c-Src and SrcYF also effectively tyrosine-phosphorylated ectopically expressed Vav2. To confirm that the kinase activity of Src was required for the tyrosine phosphorylation of Tiam1 and Vav2 by Src, we used an inactive form of SrcYF (SrcYF-KM). Neither Tiam1 nor Vav2 were phosphorylated when cotransfected with SrcYF-KM. Together, these results indicate that Src can phosphorylate Tiam1 on tyrosine residues and that this effect is dependent on the kinase activity of Src.

Next, we took advantage of the observation that reactive
oxygen species, such as H₂O₂, activate c-Src (33) to examine whether endogenous tyrosine kinases of the Src family were able to phosphorylate Tiam1. As shown in Fig. 4A, endogenous Tiam1 was tyrosine-phosphorylated upon H₂O₂ treatment. In the presence of H₂O₂, orthovanadate is oxidized to pervanadate, which increases protein tyrosine phosphorylation because of its inhibitory effect on protein-tyrosine phosphatases (34, 35). Interestingly, the combined use of low concentrations of H₂O₂ and vanadate, which had no effect when added alone, induced a strong phosphorylation of Tiam1. As an approach to determine whether this phosphorylation was mediated, at least in part, by members of the Src family, the cells were preincubated with the Src inhibitors PP2 and SU6656, the latter being a newly characterized compound with a high specificity toward Src kinases (28). Both compounds partially inhibited the tyrosine phosphorylation of Tiam1 induced by pervanadate (Fig. 4B). Collectively, these results suggest that in addition to v-Src, endogenous tyrosine kinases of the Src family can phosphorylate the Rac1 GEF Tiam1.

Tiam1 exhibits a number of structural domains, an N-terminal PH domain, a Ras-binding domain, a PDZ domain, and a C-terminal DH/PH domain (reviewed in Ref. 21). To delineate the regions of Tiam1 that are phosphorylated by Src, we made use of various truncated mutants of Tiam1 and examined them for tyrosine phosphorylation in HEK 293T cells transfected with active Src. The expressed constructs included the first 750 amino acids and the N-terminal PH domain (TiamN750); a C-terminal fragment containing the rest of the molecule including the Ras-binding domain, PDZ, and DH/PH domain (TiamC841); and a construct that consisted of the last 580 amino acids, including the DH/PH domain (TiamC580). As shown in Fig. 5, the three fragments were all effectively phosphorylated in tyrosine residues by Src. These data indicated that Tiam1 contains multiple phosphor-acceptor sites that are susceptible to Src-induced tyrosine phosphorylation.

**Src Potentiates Tiam1-induced Rac1 Activation and Promotes Rac1-dependent Signaling**—We next asked whether Src is able to regulate Tiam1 function. First, we determined whether Src and Tiam1 could cooperate to stimulate the accumulation of activated, GTP-bound Rac1 in vivo. For these experiments, we expressed in HEK 293T cells limited amounts of cDNA for SrcYF and Tiam1, which induced only a slight activation of Rac1 when expressed alone. When Tiam1 was co-transfected with SrcYF, we detected a strong potentiation of Rac1 activation (Fig. 6). As expected, SrcYF also strongly potentiated the activation of Rac1 by Vav2. Of interest, the stimulatory effect of active Src on the in vivo GEF activity of Tiam1 was also reflected in the ability of SrcYF to promote morphological and transcriptional events regulated by Rac1. As an approach to study the effects of Src on cell morphology, we used PAE cells, in which Rho GTPases promote characteristic morphological changes by controlling a variety of actin-based cytostructures (36). Indeed, as shown in Fig. 7, in these cells activated forms of RhoA (RhoAQL) induce the formation of membrane ruffling and lamellipodia formation (arrows) in 38% of transfected cells, as judged by GFP expression. The pictures are representative of three independent experiments. At least 10 high power fields were analyzed for each condition.
was abolished by a dominant negative form of Rac1 (Rac1 N17), whereas a dominant negative form of Cdc42 (Cdc42 N17) had only a limited effect (less than 20% of inhibition) (data not shown). This suggests that the activating effect on JNK exerted by SrcYF and Tiam1 is specifically mediated by Rac1.

Finally, to test whether the cooperation of Tiam1 and SrcYF could also have effects on gene expression regulation, we performed a gene reporter assay using NIH 3T3 cells transfected with the c-Jun promoter followed by a luciferase gene. Consistent with the activation of the JNK pathway, the combination of both Tiam1 and SrcYF induced a strong potentiation of the activation of the c-Jun promoter (Fig. 8B). Together, our results indicate that SrcYF can cooperate with Tiam1 to induce the activation of Rac1 and the consequent stimulation of downstream signaling pathways regulated by this small GTPase.

**DISCUSSION**

Although activated Src can stimulate the Ras-ERK pathway (8), cells transformed by v-src often exhibit only a limited increase in the levels of active Ras and ERK1/2 (9, 11), which suggests that this potent oncogene may utilize signaling routes in addition to or other than the ERK pathway to exert its remarkable biological activity. Indeed, Src can stimulate c-myc expression in a Ras-ERK-independent manner (10), a process that is also required for Src-induced transformation (38). Other members of the mitogen-activated protein kinase superfamily, such as the c-Jun N-terminal kinase (JNK) and p38, have also been reported to be highly activated in v-src-transformed NIH 3T3 fibroblasts and to play a major role in the phosphorylation and activation of STAT3, a transcription factor that is also inducible by SrcYF and Tiam1 is specifically mediated by Rac1.

Of interest, many of the biochemical routes stimulated by Src overlap with those regulated by Rac1. In fact, this small GTPase of the Rho family is required for the ability of activated Src to stimulate c-myc expression (10). In line with this observation, we found that cells transformed by v-src exhibit remarkable increased levels of GTP-bound Rac1 in vivo, which is aligned with the recently reported increase in Rac-GTP levels in neuroretina cells expressing a temperature-sensitive mutant of v-src (11). These observations may help explain why v-src-induced foci resemble more closely the punctuated morphology of foci induced by constitutively activated Rho GTPases and active guanine-nucleotide exchange factors rather than the more diffuse morphology displayed typically by Ras and the active forms of Raf and MEK (29, 30). Furthermore, inhibition of Rac1 function dramatically diminished the ability of v-src to transform NIH 3T3 cells, collectively supporting that this GTPase is an integral component of the transforming pathway elicited by v-src.

Several GEFs for small GTPases have been found to be regulated by tyrosine phosphorylation, including the three members of the Vav family, proto-Dbl, Ras-GEF, and the RGS-containing GEFs for Rho PDZ-RhoGEF and LARG (22–24, 39–41). Among them, Vav1 is the best example of a GEF that is tightly regulated by tyrosine phosphorylation (22, 23). However, Vav1 is predominantly expressed in hematopoietic cells (42). Vav2, a protein structurally related to Vav1, exhibits enhanced GEF activity toward Rac1, RhoA, RhoG, and Cdc42 (24, 43, 44) and can also be regulated by tyrosine phosphorylation (24). Because Vav2 is ubiquitously expressed, this GEF represents a likely candidate to mediate the activation of Rho,
GTPases by Src (42). However, we have recently observed that the ability of Src to stimulate c-myc expression by a Rac1-dependent pathway in NIH 3T3 cells can be only partially explained by the activation of Vav2 (10). Indeed, expression of Vav2 in NIH 3T3 cells is limited, and we and others have shown that tyrosine phosphorylation of endogenous Vav2 is detectable in NIH 3T3 cells exposed to PDGF treatment upon immunoprecipitation of large amounts of cellular proteins (10, 25).

The family of Rac1 GEFs has extended dramatically in the last years (reviewed in Ref. 21). Among them, Tiam1 is one of the GEFs exhibiting the highest specificity for Rac1. Tiam1 is widely expressed, and detectable expression levels of Tiam1 are phosphorylated upon integrin activation, despite the ability of Vav2 in NIH 3T3 cells is limited, and we and others have shown that tyrosine phosphorylation of endogenous Vav2 is detectable in NIH 3T3 cells (results not shown). Thus, our results showing that v-Src and active c-Src can phosphorylate and potentiate Tiam1-induced Rac1 activity may explain the activation of Rac1 by Src in several tissues and cell lines. In addition, the fact that oncogenic Src phosphorylates Tiam1 suggests that tyrosine phosphorylation may be a novel mechanism of regulation of this GEF. Thus far, Tiam1 has been shown to be regulated by the activity of Rac. However, the nature of the activating mechanism(s) acting upstream from Src can determine the choice of the Src downstream targets. For example, phosphorylation of Vav2 by Src is a critical component of the pathway by which PDGF receptors activate Rac1 (10), but unlike Vav2, Tiam1 is not tyrosine-phosphorylated in response to PDGF in NIH 3T3 cells (results not shown). Conversely, Vav2 is not tyrosine-phosphorylated upon integrin activation, despite the ability of Tiam1 to promote the activation of its GEF activity in vivo is at the present unknown. Tyrosine phosphorylation of Tiam1 may control the activity of its regulatory proteins and, alternatively, may activate the intrinsic catalytic exchange activity of this GEF. We can envision that future studies exploring the mechanism by which Src-dependent tyrosine phosphorylation of Tiam1 affects its activity will provide further insights into how oncogenic alleles of Src activate Rac1, thereby promoting cell transformation.

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