Stat3 Plays an Important Role in Oncogenic Ros- and Insulin-like Growth Factor I Receptor-induced Anchorage-independent Growth*

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The role of signal transducers and activators of transcription (STATs)1 in receptor protein-tyrosine kinase (PTK)-induced cell growth and transformation was investigated using an inducible epidermal growth factor receptor-Ros chimeric receptor called ER2 and a constitutively activated insulin-like growth factor I receptor called NM1, both of which are able to induce anchorage-independent growth of NIH 3T3 cells. ER2 and NM1 receptor PTKs are able to cause Stat3 activation. Co-expressing the dominant negative Stat3 mutant with ER2 or NM1 in transiently or stably transfected cells resulted in a dramatic inhibition of colonies induced by these receptor PTKs and a moderate inhibition of their mitogenicity in monolayer. Therefore, Stat3 is not only important for initiation of transformation, as demonstrated by inhibition of the epidermal growth factor-inducible colony formation of the ER2 cells by the mutant, but it is also required for the maintenance of transformation, as evidenced by reversion of the NM1 transformed cells. The DNA binding and transcriptional activities of the endogenous Stat3 were greatly inhibited in the ER2 and NM1 cells co-expressing the Stat3 mutants. We conclude that activated function of Stat3 is required for the establishment and maintenance of Ros and insulin-like growth factor I receptor PTK-induced cell transformation.

Signal transducers and activators of transcription (STATs)1 were originally discovered as transcription factors involved in interferon-induced gene expression (1, 2). The STATs were subsequently shown, also, to be involved in mediating signal transduction of a large number of cytokines that are important for differentiation and maturation of immune cells. Binding of a type I or type II cytokine to its cognate receptor results in its dimerization and recruitment and activation of a specific tyrosine kinase(s) of the Janus kinase (JAK) family. Tyrosine phosphorylation of the cytoplasmic domain of the cytokine receptor generates sites for the binding of STAT proteins via their SH2 domain. Subsequent tyrosine phosphorylation at the carboxyl domain of STATs catalyzes the dimerization, which is followed by translocation to nucleus, and functions as an activated transcriptional factor. To date, seven STATs (1–6, including 5a and 5b) and four JAKs (1–3 and Tyk2) have been identified in mammalian cells (1, 2). Differential expression and activation of specific cytokine receptors, JAKs, and STATs in different types of cells constitutes the complexity and specificity of the JAK-STAT-mediated signaling and gene regulation in those cells. Recent studies indicate that STATs could also function as effectors for a variety of growth factor receptors, including epidermal growth factor receptor (EGFR) (3–6), platelet-derived growth factor receptor (6–8), colony-stimulating factor I receptor (6, 7, 9), and insulin receptor (10–12). In the case of growth factor receptor-induced tyrosine phosphorylation of STATs, accumulating evidences suggest that JAKs may not be required (7, 13, 14). These observations suggest that STATs may also play a role in receptor protein-tyrosine kinase (RPTK)-mediated functions, including cell growth and differentiation. Moreover, STATs have also been implicated in either promoting or preventing growth arrest and apoptosis (15–18). For example, Stat1 is required for EGF-induced expression of cyclin inhibitor p21(crip) and growth arrest of A431 cells (19), whereas Stat3 functions in preventing apoptosis of pre-B cells (18). Overexpression of insulin-like growth factor I receptor (IGFR) was shown to be able to protect 32D cells from apoptosis upon interleukin 3 withdrawal, and this protection appears to correlate with sustained de novo synthesis of Stat1 and Stat5 (20).

More recently, STATs were found to be activated in certain tumors and oncogene-transformed cell lines (21–31). Stat3 is most prominently activated in src-transformed NIH 3T3 cells (21, 30), whereas Stats 1, 3, and 5 are activated in src-transformed myeloid cells (23). Stat3 is also constitutively activated in v-sis and v-fps-transformed fibroblasts (24). The oncogenic v-abl and Eyk PTKs induce activation of Stats 1, 5, and 6 in pre-B cells (25) and Stats 1 and 3 in chicken embryo fibroblasts (31), respectively. Furthermore, specific members of STATs were found to be activated in various human tumors and cell lines derived from them (24, 26, 28, 30, 32, 33). These observations raised yet another possible function of STATs, namely in oncogenesis. However, direct evidence for the involvement of STATs in oncogene-induced cell transformation has only very recently begun to emerge. Using a dominant negative mutant of Stat1, Zong et al. (31) suggested that it plays a very important role in EryK-induced transformation of NIH 3T3 cells. This observation, together with previously reported activation of specific STATs by v-src and v-abl, further strengthens the possibility that STATs plays some important role in oncogenic PTK-induced cell transformation.

Our laboratory has been interested in exploring the mechanism of cell transformation by oncogenic RPTKs Ros, insulin receptor, and IGFR. v-ras is an oncogenic gag-Ros fusion receptor gene spontaneously transduced by an avian leukemia virus
(34). We have constructed an EGFR-Ros chimeric receptor and shown that it is capable of inducing EGF-dependent transformation of NIH 3T3 cells (35). We have also engineered a constitutively oncogenic gag-IGFR fusion receptor called NM1 and demonstrated its transforming ability in chicken embryo fibroblasts (36) and NIH 3T3 cells. Using a temperature-sensitive v-ros mutant, ligand-inducible EGFR-Ros chimeras, and the constitutively activated NM1 gag-IGFR, we have previously observed cell type-specific activation of STAT proteins in chicken embryo fibroblasts and NIH 3T3 cells by Ros and IGFR. To further explore the role of Stat3 in Ros and NM1-induced cell transformation, we have employed dominant negative (dn) mutants of Stat3 to investigate its role in the establishment and maintenance of cell transformation by these oncogenic RPTKs. We report here that both Ros and NM1 activate predominantly Stat3 in NIH 3T3 cells and that Stat3 is required for Ros- and NM1-induced anchorage-independent growth, whereas Stat3 is less critical for the ability of these RPTKs to stimulate cell growth in monolayer. During the course of this study, two reports were published demonstrating that Stat3 plays an essential role in v-src-induced transformation of NIH 3T3 cells. (38, 39). Overall, these studies provide initial direct evidence for the role of STATs in cell transformation and oncogenesis.

**EXPERIMENTAL PROCEDURES**

**Cell Culture—**NIH 3T3 fibroblasts were grown in Dulbecco’s modified Eagle’s medium (DMEM) containing 5 or 10% bovine calf serum.

**DNA Transfection—**Transfection were carried out by standard calcium phosphate method (40, 41). Cells were seeded at 5 × 10⁴/100-mm-diameter dish in DMEM plus 10% calf serum at 18–24 h prior to transfection. Total DNA for transfection was about 10–20 μg of appropriate plasmids per dish plus 20 μg of calf thymus DNA. Transfection was terminated 15 h later by removing the medium and washing twice with fresh medium, and cells were then maintained in the same medium. Stable clones were selected in growth medium containing 400 μg/ml G418 (Geneticin, Life Technologies, Inc.), 200 μg/ml hygromycin B (Sigma), or 2 μg/ml puromycin (Sigma), depending on the plasmid used, 48 h after transfection.

**Plasmids Used—**pECE-ER2 is an EGFR-Ros chimeric receptor construct that induces transformation of NIH 3T3 cells in response to EGF as described previously (35). pEFP2 and pMV12ER2 were generated by removing the EGFR-Ros insert from the pECE-ER2 construct (35), and it was cloned into the human elongation factor promoter-based neomycin plasmid pEF3 and the Moloney leukemia virus long terminal repeat-driven, hygromycin-resistant plasmid pMV12, separately (42). dnStat3-1 contains a mutation of Glu434-Glu435 to Ala-Ala (43), and dnStat3 contains the same Glu-Glu to Ala-Ala (43), and dnStat3-2 contains the same Glu-Glu to Ala-Ala and Val663,Val665 to Ala-Ala-Ala mutation (39) in the DNA binding domain of Stat3. Both were cloned in the cytomegalovirus promoter-based expression vector pRcCMV (39, 43) and were kindly given to us by Drs. James Darnell and Curt Horvath. Two reporter plasmids were used: pLucTKS3(38), which contains multimerized Stat3-specific binding sites derived from the human C-reaction protein gene inserted upstream of the TK minimal promoter (kindly given to us by Dr. Richard Jove), and Ly6ELuc (44), which contains three GAS sites from the promoter of the Ly6E gene (kindly given to us by Dr. Curt Horvath). CMV-β-gal plasmid was purchased from Invitrogen.

**Biological Assay—**Cell transformation was monitored by anchorage-independent growth in soft agar medium as described previously (45–47). If EGF was included in the assay, EGF containing soft agar medium was used for top agar overlay, and DMEM containing EGF was added on top every 5 days. The control dishes were overlaid with regular soft agar medium and subsequently fed with regular DMEM containing 5% calf serum.

**Protein Analysis—**Protein extraction, immunoprecipitation, and Western blotting were done according to published procedures (45–47). Anti-Stat1 (E-23), anti-Stat3 (C-20), anti-Stat5b (C-17), and anti-Flag (D-8) antibodies were purchased from Santa Cruz Biotechnology. Anti-P-Tyr (RC20) was purchased from Signal Transduction Laboratories.

**Electrophoretic Mobility Shift Assay (EMSA)—**Cultures of stable transfectants or cells 48 h after transfection were starved for 20 h in serum-free DMEM and then stimulated with EGF for 15 min. Whole cell lysates were prepared from the control and EGF-treated cells. Briefly, after washing twice with phosphate-buffered saline, cells were harvested in 1 ml phosphate-buffered saline and pelleted by centrifugation. Cells were resuspended in twice the pellet volume of high salt buffer (20 mM Hepes, pH 7.9, 20 mM NaF, 1 mM NaPPi, 1 mM Na₃VO₄, 1 mM EGTA, 1 mM EDTA, 1 mM dithiothreitol, 400 mM NaCl, 20% glycerol, 0.1% Triton X-100, 1 mM phenylmethylsulfonyl fluoride, 1%
Trasylol, and 1 μg/ml leupeptin) and were rocked for 30 min at 4 °C. The lysates were centrifuged at 15,000 × g for 20 min at 4 °C, and protein concentrations of the clarified lysates were determined by the Bradford assay. Cytosolic and nuclear fractions were prepared as described (5, 48). The M67 serum inducible element (SIE) (49) oligonucleotide containing Stat1 and Stat3 binding sites was labeled with [α-32P]dGTP by Klenow end filling and purified by electrophoresis in a 12% nondenaturing gel with a bis:acrylamide ratio of 1:19. The β-CAS oligodeoxynucleotide probe contains the Stat5 binding sequence derived from the rat β-casein gene promoter (50). DNA binding was performed by adding 15–20 μg of protein lysate to 18 μl of reaction mixture containing 65 mM NaCl, 10 mM Hapes, pH 7.9, 1 mM dithiothreitol, 2% Ficoll 400, 4% glycerol, and 1 μg of poly(dI-dC) (Amersham Life Science Inc.); the mixture was preincubated on ice for 15 min; and then, approximately 30,000 cpm (1–1.5 ng) of the oligodeoxynucleotide was added to the reaction mixture, which was further incubated for 30 min at room temperature. The samples were then analyzed in a 20 × 20-cm 5% polyacrylamide gel with a bisacrylamide ratio of 1:39 containing 2.5% glycerol and 0.5 × TBE (45 mM Tris base, 1 mM EDTA). The electrophoresis was carried out at 175 V until the faster migrating bromphenol blue dye was about 4 cm from the bottom edge. The gel was dried and subjected to autoradiography using Kodak XAR5 film for 1–3 days with intensifying screens at −70 °C.

**RESULTS**

**Tyrosine Phosphorylation and Activation of STAT Proteins in ER2- and NM1-expressing Cells**—To determine whether STAT proteins are activated by the oncogenic RPTKs Ros and gag-IGFR (NM1), we expressed a ligand-inducible Ros and the constitutively activated NM1 gag-IGFR in NIH 3T3 cells and analyzed for tyrosine phosphorylation and DNA binding activity of STAT proteins. Activation of the ER2 Ros chimera by EGF led to significant increase in tyrosine phosphorylation of Stat3 (Fig. 1B) This increased tyrosine activation was correlated with increased DNA binding activity of Stat3 (Fig. 1D). EMSA analysis revealed increase in Stat3 homodimer (SIFA) and Stat3/Stat1 heterodimer (SIFB) binding to the SIE probe upon Ros activation in both cytosolic and nuclear fractions, although the latter was more prominent (Fig. 1D). There was a basal level of Stat1 tyrosine phosphorylation that was largely insensitive to EGF stimulation (Fig. 1A). In contrast, tyrosine phosphorylation of Stat5b was greatly increased upon EGF treatment in control and ER2-expressing cells (Fig. 1C), implying its activation via endogenous EGFR. This result indicates that activation of Ros does not lead to further activation of Stat5 beyond the level of activation in the control cells. We conclude that Ros is able to specifically activate Stat3 in NIH 3T3 cells.

Similar experiments were performed in NM1-expressing NIH 3T3 cells. Tyrosine phosphorylation of Stat3 was most significantly increased by NM1 gag-IGFR (Fig. 2A). Little effect on Stat1 was observed. Stat5b tyrosine phosphorylation was also increased. DNA binding and mobility shift assay using SIE and β-CAS probes confirmed the activation of Stat3 and Stat5 (Fig. 2B). Although prominent activation of Stat3 was repeatedly observed, the extent of Stat5 activation was not as significant in other independent experiments (data not shown). Therefore, NM1 is capable of reproducibly activating Stat3 in NIH 3T3 cells.

**dnStat3 Mutants Inhibit Ros- and NM1-induced Cell Transformation**—Because both Ros and NM1 PTKs are able to activate Stat3 signaling, we next investigated its potential role in Ros- and NM1-induced cell growth and transformation. For this purpose, we employed the approach of dominant negative mutants to block the endogenous Stat3 function. The Stat3 mutants used here are defective in DNA binding and have been shown to be able to block the wild type Stat3 activity, presumably by forming a nonproductive heterodimer (43). Dominant negative Stat3 mutants were introduced into the ER2- or NM1-expressing cells. Stable clones co-expressing the oncogenic PTK and a mutant Stat3 were selected using the appropriate drugs. Fig. 3 compares the colony-forming abilities of clones expressing ER2 alone or co-expressing ER2 and the dn mutants. dnStat3-expressing cells formed significantly fewer and smaller colonies compared with the control vector transfected parental ER2 cells. The control and dnStat3-expressing lines...
expressed equivalent amounts of ER2 protein, whereas the
dnStat lines expressed about a 2-fold excess of dnStat3 protein
(Fig. 3D). In addition, the ER2 chimeric receptors in all of those
clones are equally responsive to EGF stimulation. Upon long-
term passages of those stable lines, we observed that some of
the clones displayed a reduced expression level of dnStat3 with
concurrent loss of inhibition on colony formation (data not
shown). Furthermore, the inhibition of Ros-induced colony for-
mation by dnStat3 was also assessed by the transient trans-
fection method (Fig. 4). Three independent experiments were
carried out by transiently transfecting the dnStat3 mutant into
ER2-expressing cells, which were assayed for colony formation
48 h later. An average of 50% inhibition on colony formation
was observed (Fig. 4). Fig. 4B shows ER2 and dnStat3 protein
expression; shown here is a representative one of three exper-
iments. The inhibition by dnStat3 was not due to apoptosis or
decreased cell viability, as no reduction in drug-resistant colo-
nies were observed when the transfected cells were subjected to
drug selection (data not shown).

Similar experiments were carried out with NM1 by trans-
fecting dnStat3 mutants or control empty vector into the stable
NM1-expressing 3T3 cells. Mass stable cultures expressing
only the NM1 or co-expressing NM1 and either of the dn mu-
tants were prepared. A result similar to that of Ros was ob-
tained. The dnStat3-expressing cells formed fewer and smaller
colonies (Fig. 5, A–C), although the size of colonies appeared to
be more significantly affected than the number of colonies. Ag-
ain, the control and dnStat3-expressing lines express about
equal amount of NM1 protein (Fig. 5D). The dnStat3-1-express-
ing cells had about a 2-fold excess of total Stat3 protein, and the
dnStat3-2 transfectant expressed the FLAG-tagged dnStat3-2
protein as expected (Fig. 5, E and F). Furthermore, transient
transfection of 3T3 cells with 5 µg of the NM1 plasmid, to-
gether with control or dnStat mutant plasmid in a ratio of 1:3,
showed that dnStat3-1 and dnStat3-2 inhibited the NM1 colo-
y-forming activity by 52% and 62%, respectively.

![Fig. 3. Inhibition of ER2-induced colony formation by the dnStat3-1 mutant in stable expressing lines. A and B, three independent stable NIH 3T3 lines expressing ER2 alone and three lines co-expressing ER2 and dnStat3-1 were assessed for their colony-forming ability.](image1)

![Fig. 4. Inhibition of colony-forming ability of the ER2-expressing lines transiently transfected with dnStat3-2. Three independent ER2-expressing 3T3 lines were transfected with either 10 µg of control (RcCMV) or dnStat3-2 plasmid. 48 h later, cells were used for colony formation assay. Histograms in A represent the average values of three experiments. B, parallel culture in each transfection experiment was subjected to analysis of the ER2 and dnStat3-2 protein expression. 10 µg each of protein extracts was subjected to direct Western blotting with the indicated antisera, Stat3-2 is Flag-tagged (39). The result from one of the experiments is shown here.)](image2)
To assess whether inhibition of the anchorage-independent growth by dnStat3 mutants was due to its general inhibition on mitogenicity, we performed assays of cell growth in monolayer culture (Fig. 6). Three independent dnStat3-1-expressing ER2 lines and two control parental ER2 lines were compared for their growth rate in 5% calf serum plus 50 ng/ml EGF. The dnStat3-expressing cells appeared to have a slower rate of growth during the initial phase, resulting in 30–40% fewer cells at the end point. A similar result was obtained with mass cultures expressing NM1 alone or co-expressing NM1 and the dnStat3-1 mutant (Fig. 6B). Here, we observed a 20–30% reduction of the numbers of cells during the later time points. The observed growth inhibition of the ER2 and dnStat3 co-expressing cells in monolayer cannot fully account for their dramatic reduction in both number and size of colonies in soft agar, whereas the growth inhibition of the NM1 and dnStat3 co-expressing cells is more in accordance with their reduced colony size in soft agar. We conclude that the dnStat3 mutant is able to block the Ros-induced establishment of cell transformation and revert the transformed state of NM1-expressing cells, as reflected by anchorage-independent growth. The inhibition of ER2-induced colonies cannot be fully accounted for by general inhibition of mitogenicity by the dnStat3. Our results imply that the Stat3 signaling function(s) plays a differential role in Ros-induced anchorage-independent growth versus cell growth in monolayer.

**ER2- and NM1-induced Stat3 Activation Is Blocked by Dominant Negative Stat3**—To further characterize the inhibition of colony formation by dnStat3, the DNA binding and transcriptional activities of Stat3 were examined. First, we compared the DNA binding activity of Stat3 proteins obtained from the ER2 lines and two control parental ER2 lines were compared for their growth rate in 5% calf serum plus 50 ng/ml EGF. The dnStat3-1-expressing cells appeared to have a slower rate of growth during the initial phase, resulting in 30–40% fewer cells at the end point. A similar result was obtained with mass cultures expressing NM1 alone or co-expressing NM1 and the dnStat3-1 mutant (Fig. 6B). Here, we observed a 20–30% reduction of the numbers of cells during the later time points. The observed growth inhibition of the ER2 and dnStat3 co-expressing cells in monolayer cannot fully account for their dramatic reduction in both number and size of colonies in soft agar, whereas the growth inhibition of the NM1 and dnStat3 co-expressing cells is more in accordance with their reduced colony size in soft agar. We conclude that the dnStat3 mutant is able to block the Ros-induced establishment of cell transformation and revert the transformed state of NM1-expressing cells, as reflected by anchorage-independent growth. The inhibition of ER2-induced colonies cannot be fully accounted for by general inhibition of mitogenicity by the dnStat3. Our results imply that the Stat3 signaling function(s) plays a differential role in Ros-induced anchorage-independent growth versus cell growth in monolayer.

**Effect of Stat3 on Ros- and IGFR-induced Transformation**

**Fig. 6.** Effect of the dnStat mutant on growth rate of the ER2- and NM1-expressing cells. A, two control plasmid-transfected ER2-expressing lines (PB1 and PB4) and three ER2 lines co-expressing ER2 and dnStat3-1 (dnst2, dnst6, and dnst9) were compared for their growth rate in DMEM containing 5% serum. 1 × 10^5 cells were seeded per 60-mm dish at the beginning of the assay. Duplicate dishes were counted for cell numbers at each time point. B, a stable NM1-expressing 3T3 line was transfected with control or dnStat3-1 plasmid, and drug-resistant clones were selected as in Fig. 5 and pooled to form the control NM1-expressing mass culture (NM1 PB) and the dnStat3-1 co-expressing mass culture (NM1 dnst). The growth rate assay was similar to that shown in A.
overnight followed by EGF stimulation. The cells were starved in serum-free medium overnight and then with or without EGF treatment for 15 min. 20 μg of each total cell lysate was subjected to DNA binding by EMSA using the M67 SIE probe as described in Fig. 1. B, NIH 3T3 cells were transfected with control (phEF), pER2ER2 (5 μg each), or pER2ER2 plus dnStat3-2 (20 μg) plasmid. 24 h later, cells were changed to serum-free medium overnight followed by EGF stimulation. 20 μg each of the total cell lysates was subjected to DNA binding analysis as described in A. C, 3T3 cells were transfected with ER2 plus wild type Stat3 or with ER2 plus wtStat3 and dnStat3-2 and analyzed as described in B.

FIG. 7. Inhibition of ER2-induced activation of the Stat3 DNA binding activity by dnStat3 mutants. A, a control ER2-expressing line (C, I) and two dnStat3-1 co-expressing ER2 lines (dnStat3-1, 1 and 2) were analyzed for activation of the DNA binding activity of the endogenous Stat3 upon activation of Ros by EGF. The cells were starved in serum-free medium overnight and then with or without EGF treatment for 15 min. 20 μg of each total cell lysate was subjected to DNA binding by EMSA using the M67 SIE probe as described in Fig. 1. B, NIH 3T3 cells were transfected with control (phEF), pER2ER2 (5 μg each), or pER2ER2 plus dnStat3-2 (20 μg) plasmid. 24 h later, cells were changed to serum-free medium overnight followed by EGF stimulation. 20 μg each of the total cell lysates was subjected to DNA binding analysis as described in A. C, 3T3 cells were transfected with ER2 plus wild type Stat3 or with ER2 plus wtStat3 and dnStat3-2 and analyzed as described in B.

The precise role of Stat3 in RPTK-induced cell transformation remains an intriguing and challenging question. The evidence for its involvement in cell transformation is consistent with various published observations for its functioning in growth and protection from apoptosis (15, 18). Because the only known function of STATs is their role in transcriptional regulation, it is logical to assume that modulation of a certain gene(s) by Stat3 is essential for those PTK oncogene-induced cell transformation. It has been shown that Stat3 is not activated in v-Ras transformed cells and that dnStat3 has no effect on Ras-induced transformation of NIH 3T3 cells (38, 39). Therefore, Ras must be able to signal through a Stat3-independent pathway to modulate those target genes. Alternatively, Ras may be capable of activating other genes with functions equivalent to the Stat3 target genes induced by those PTK oncogenes. In either case, modulation of those genes must be via a Ras-specific signaling pathway that is Stat3-independent and is not shared by the Src, Ros, and IGFR PTK oncogenes. Much is still to be learned about the activation and down-regulation of specific genes required for initiation and maintenance of cell transformation. A requirement of Stat3 for

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**DISCUSSION**

Our studies indicate that activation of Stat3 by Ros and NM1 plays an important role in establishment and maintenance of cell transformation by these oncogenic receptor PTKs. Stat3-mediated signaling appears to be more critical for anchorage-independent growth than for cell growth. It is likely that certain adhesion-triggered signaling can be fulfilled by Stat3-mediated function when cells are grown in soft agar. Together with the studies by Zong et al. (31), Turkson et al. (38), and Bromberg et al. (39), we have provided the initial direct evidence for the involvement of Stat3 in the process of cell transformation by oncogenic PTKs.

Although the mechanism by which various cytokines induce activation of STATs proteins has been well characterized through the use of JAK knockout cell lines (51-53), the mechanism for STATs activation via growth factors that signal through RPTKs is less clear. Recent evidence has suggested that JAKs may not be essential in growth factor receptor-mediated activation of STATs (7, 13, 14). The v-Src protein has been shown to be associated in a complex with Stat3, implying that Stat3 can be directly phosphorylated by v-Src (21). We have recently reported that the cytoplasmic domain of insulin receptor could interact with Stat5 in a yeast two-hybrid assay and that this interaction appears to require PTK activity of insulin receptor (10). Moreover, Stat5 can be tyrosine-phosphorylated by purified insulin receptor PTK in vitro (10). Although those observations strongly suggest that RPTKs may be able to directly interact and phosphorylate STATs, the possibility of indirect activation via JAKs is not excluded. JAK family kinases were shown to be constitutively activated in Src-transformed fibroblasts (54). The two mechanisms of STATs activation are not mutually exclusive, and they may actually coexist and operate in parallel in oncogenic PTK-transformed cells.

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were grown in soft agar medium and that the defectiveness can be rescued by transfection with a cyclin A expression vector (37). In this instance, the revertant cells are impaired in expression of cyclin A when they are deprived of adhesion. It is possible that activation of Stat3 by those oncogenic PTKs may allow this transcriptional factor to induce the expression of genes, such as cyclins, that are required for growth in the absence of adhesion.

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