Activation of STAT3 by the c-Fes Protein-tyrosine Kinase*

Received for publication, July 14, 1997, and in revised form, November 21, 1997

Kristie L. Nelson‡, Jim A. Rogers‡, Tammy L. Bowman§, Richard Jove§, and Thomas E. Smithgall¶

From the ‡Eppley Institute for Research in Cancer and Department of Biochemistry and Molecular Biology, University of Nebraska Medical Center, Omaha, Nebraska 68198-6805, and the §Molecular Oncology Program, H. Lee Moffitt Cancer Center and Research Institute, University of South Florida College of Medicine, Tampa, Florida 33612

STATs (signal transducers and activators of transcription) are transcription factors that contain SH2 domains and are activated by tyrosine phosphorylation, often in response to cytokine stimulation. Recent evidence indicates that the transforming tyrosine kinases encoded by the v-Src, v-Abl, and v-Fps oncogenes can induce STAT activation, suggesting that their normal cellular homologs may contribute to STAT activation under physiological conditions. In this report, we provide direct evidence that c-Fes, the normal homolog of v-Fps, potently activates STAT3. Transient transfection of human 293T cells with STAT3 and Fes resulted in strong stimulation of STAT3 DNA binding activity. In contrast, only modest activation of STAT5 by Fes was observed in this system, indicative of possible selectivity. To determine whether Fes-induced STAT3 activation is dependent upon endogenous mammalian kinases, co-expression studies were also performed in SF-9 insect cells. Fes also induced a dramatic increase in STAT3 DNA binding activity in this system, whereas no activation of STAT5 was observed. As a positive control, both STAT3 and STAT5 were shown to be activated by the Bcr-Abl tyrosine kinase in SF-9 cells. Fes induced strong tyrosine phosphorylation of STAT3 in both expression systems, consistent with the gel-shift results. Fes and STAT3 have been independently linked to myeloid differentiation. Results presented here suggest that these proteins may cooperate to promote differentiation signaling in response to hematopoietic cytokines.

STATs (signal transducers and activators of transcription) are a class of transcription factors with SH2 domains that are activated in response to tyrosine phosphorylation. STATs are often activated by members of the JAK family of protein-tyrosine kinases in response to cytokine stimulation. The activation mechanism involves SH2-dependent recruitment of the STATs to tyrosine-phosphorylated cytokine receptors. The STATs are then phosphorylated by receptor-associated JAKs, which induce dimerization via reciprocal SH2-phosphotyrosine interaction. The STAT dimers then enter the nucleus and bind specific DNA elements, leading to transcriptional activation. The JAK-STAT pathway is the subject of many recent comprehensive reviews (1–5).

In addition to JAKs, other classes of non-receptor protein tyrosine kinases have recently been shown to induce STAT activation. For example, STAT3 is constitutively activated in fibroblasts transformed with v-Src (6, 7). Src forms a stable complex with STAT3, suggesting that STAT3 activation may be direct (7). However, more recent work shows that JAK1 and to some extent JAK2 are activated in Src-transformed cells, suggesting that JAKs may contribute to STAT activation by Src kinases (8). Another study has shown that STATs 1 and 5 are activated in fibroblasts transformed by v-Abl (9). In this case, v-Abl was shown to form stable complexes with endogenous JAK kinases, suggesting that STAT activation by v-Abl may involve JAKs as well. Related studies with chronic myelogenous leukemia cell lines transformed by Bcr-Abl show a similar pattern of STAT activation, with some evidence for JAK involvement (10–12).

Recently, we observed that fibroblast transformation by v-Fps correlates with potent activation of endogenous STAT3 (13). This finding suggested that STAT3 may also be a substrate and effector protein for c-Fes, the normal human homolog of v-Fps (14–16). The c-Fes tyrosine kinase exhibits strong hematopoietic expression, is activated in response to multiple cytokines (17–20), and has been linked directly to the control of myeloid differentiation (21, 22). Recent studies have shown that activation of STAT3 is necessary for induction of myeloid differentiation in response to IL-6 (23, 24), one of the cytokines known to activate c-Fes (19). Taken together, these previous studies suggest that c-Fes may contribute to STAT activation under physiological conditions. In this study, we provide evidence for the activation of STAT3 by Fes in human cells as well as SF-9 insect cells. Fes potently activated STAT3 in both systems, suggesting that STAT3 may be a direct substrate for Fes and may mediate Fes differentiation signaling in myeloid cells.

**EXPERIMENTAL PROCEDURES**

**Cell Lines—**293T cells (25) were obtained from the American Type Culture Collection (ATCC) and grown in Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum and 50 μg/ml gentamicin. SF-9 cells were obtained from the ATCC and grown in Grace's insect cell medium supplemented with 10% fetal bovine serum and 50 μg/ml gentamicin.

**Expression of Fes and STAT Proteins in 293T Cells—**The cDNAs encoding murine STAT3 and STAT5A were subcloned into the respective expression vectors pCDNA3 and pCDNA3.1(−) (Invitrogen). Construction of expression vectors for Fes and the kinase-inactive Fes mutant K590E with C-terminal FLAG epitope tags and calcium phosphate-mediated transfection of 293T cells is described elsewhere (26).

**Generation of Recombinant Baculoviruses and Expression of Proteins in SF-9 Insect Cells—**STAT3 and STAT5A cDNAs were subcloned into the baculovirus transfer vectors pVL1392 and pVL1393, respectively. The resulting constructs were used to make recombinant baculoviruses by co-transfection with BaculoGold DNA (Pharmingen) using the manufacturer's protocol. A cDNA encoding the p210 form of Bcr-Abl (generous gift of Dr. Owen Witte, Howard Hughes Medical Institute, UCLA)

*This work was supported by National Institutes of Health Grant CA58687, American Cancer Society Research Grant BE-245, and National Cancer Institute Cancer Center Support Grant P30 CA36727 to the Eppley Institute for Research in Cancer. 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: Eppley Institute for Research in Cancer, University of Nebraska Medical Center, 600 S. 42nd St., Omaha, NE 68198-6805. Tel.: 402-559-8270; Fax: 402-559-4651; E-mail: tsmithga@unmc.edu.
was used to make a recombinant baculovirus by the same procedure. Construction of baculoviruses for the expression of wild-type and kinase-defective Fes has been described elsewhere (26). Baculovirus-expressed Fes proteins carry a C-terminal FLAG epitope tag to allow for detection with the M2 monoclonal antibody. For protein expression, SF9 cells were grown to 50% confluence on 60-mm tissue culture plates and infected with recombinant baculoviruses for 1 h at 27 °C. The virus was replaced with fresh medium, and the cells were incubated for 48 h prior to preparation of cytosolic extracts.

Preparation of Cytosolic Extracts—Cytosolic extracts were made following a previously published protocol (6). Briefly, culture plates of 293T or SF9 cells were washed twice with ice-cold PBS followed by PBS containing 1 mM sodium orthovanadate and 5 mM sodium fluoride. The cells were rinsed with hypotonic buffer (20 mM HEPES, pH 7.9, 1 mM EDTA, 1 mM EGTA, 20 mM sodium fluoride, 1 mM sodium orthovanadate, 1 mM sodium pyrophosphate, 1 mM diithiothreitol, 0.5 mM phenylmethylsulfonyl fluoride, 50 μg/ml leupeptin, and 25 μg/ml aprotinin). The cells were then lysed in hypotonic buffer containing 0.2% Triton X-100 and clarified by microcentrifugation. The protein concentration of the extract was determined using the Coomassie-plus reagent (Pierce) and stored at −80 °C.

Electrophoretic Mobility Shift Assay—The probes used for STAT DNA binding included the sis-inducible element (MGFE) (27). The sequences of the SIE oligonucleotides are 5'-CTTCAATTCCTAATCCCTAAAGCT-3' and 5'-AGCTTTAGGGTGATTACGAAATGGA-3'. The sequences of the MGFE oligonucleotides are 5'-AGATTTCTAGAATTTCA-3' and 5'-GATTGAAAATCTCCTGA-3'. Oligonucleotides (20 pmol) were annealed in 10 μl of TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) by heating to 70 °C and slowly cooling to room temperature. The probes were labeled by combining 1 μl of annealed oligonucleotide with 2 μl of Labeling Mix-dATP (Pharmacia Biotech Inc.), 5 μl of a 10 mM dATP (10 mCi/ml; Amersham Life Science, Inc.), and 1 μl of the Klenow fragment of DNA polymerase (Life Technologies, Inc., 3.7 units/μl). The mixture was incubated at room temperature for 30 min. and the unincorporated nucleotides were removed by spermine precipitation (28). DNA binding reactions contained 40,000 cpm of labeled probe in a final volume of 20 μl and 2–5 μg of cytosolic protein extract. In the case of the cytosolic extract from SF9 cells, 0.5 μg of a 1.0 ml cytosolic extract from 6 × 10⁶ cells was used in each reaction. The reactions were incubated at 30 °C for 30 min and run on 5% polyacrylamide gels in 0.25X Tris borate-EDTA buffer. Gels were fixed with 10% acetic acid, 10% methanol, rinsed with water, dried, and exposed to a storage phosphor screen.

Immunoprecipitation and Immunoblotting—For immunoprecipitation, clarified cytosolic extracts were incubated with 1 μg of anti-STAT3 or anti-STAT5 antibodies (Santa Cruz Biotechnology) and 20 μg of protein G-Sepharose (50% slurry; Pharmacia) for 1 h at 4 °C. The immunoprecipitates were washed three times with 1 ml of radioimmunoprecipitation buffer (26) followed by heating in SDS-PAGE sample buffer. For immunoblotting, cytosolic extracts or immunoprecipitated proteins were run on 8% polyacrylamide gels and transferred to PVDF membranes. The membranes were incubated for 1 hour with antibodies to Fes (M2 anti-FLAG antibody; Eastman-Kodak Co.), phophotyrosine (PY20; Transduction Laboratories), STAT3 (Santa Cruz) or STAT5 (Transduction Laboratories) at 1 μg/ml in Tris-buffered saline containing 0.05% Tween-20 (TBST) and 1.5% bovine serum albumin. The membranes were then washed in TBST and probed with a secondary antibody–alkaline phosphatase conjugate in TBST-bovine serum albumin at the dilution recommended by the manufacturer (Southern Biotechnology Associates). Following further washing with TBST, the immunoreactive proteins were visualized colorimetrically using the alkaline phosphatase substrate NBT/BCIP.

RESULTS
Activation of STAT3 by c-Fes in 293T Cells—Recently we observed that endogenous STAT3 is constitutively activated in fibroblasts transformed by v-Fps, a retroviral homolog of c-Fes (13). Given the relationships of c-Fes and STAT3 to myeloid differentiation (21, 23, 24, 29), we decided to investigate whether STAT3 or other STAT factors represent possible Fes substrates and effecter molecules. To determine whether STAT3 is activated by Fes, the two proteins were expressed either alone or together in human 293T cells. Cytosolic extracts were prepared and tested for the presence of active STAT3 by gel-shift assay with an SIE probe. Control lanes include incubation of the probe in the absence of extract (SIE) and gel-shift assays of untransfected cell extracts (293T). Duplicate gel-shift assays were conducted in the presence of a 100-fold excess of unlabeled SIE to demonstrate probe specificity (COMP). The shifted complex of activated STAT3 and the SIE probe is denoted by the arrow.

Fig. 1. Activation of STAT3 by Fes in 293T cells. Human 293T cells were transfected with STAT3, Fes, or a kinase-inactive Fes mutant (Fes-KE) either alone or in the combinations shown. Cytosolic extracts were prepared and tested for the presence of active STAT3 by gel-shift assay with an SIE probe. As shown in Fig. 1, extracts from cells co-transfected with both Fes and STAT3 produced a dramatic shift in SIE mobility, indicative of STAT3 activation. Addition of a 100-fold molar excess of unlabeled SIE completely blocked complex formation, providing evidence of probe specificity.

To demonstrate that the activation of STAT3 requires the tyrosine kinase activity of c-Fes, 293T cells were co-transfected with STAT3 and a kinase-defective Fes mutant (Fes-K590E) (30). As shown in Fig. 1, co-expression with the kinase-defective protein produced only low background levels of STAT3/SIE complexes equivalent to those observed when STAT3 was expressed alone. This result shows that stimulation of STAT3 DNA binding activity requires an active Fes kinase domain and is consistent with STAT3 tyrosine phosphorylation data shown below. We also investigated the requirements for other structural features of c-Fes in STAT3 activation using a series of Fes deletion and autophosphorylation site mutants. Co-expression of STAT3 with a Fes mutant lacking the unique N-terminal domain (amino acids 1–450) (26) activated STAT3 to the same extent as wild-type c-Fes (data not shown). This result suggests that the Fes N-terminal region, while involved in the recruitment of somatostatin substra Co (31, 32) is dispensable for interaction with STAT3. However, deletion of the Fes SH2 domain or mutagenesis of Tyr-713, the major Fes autophosphorylation site (26, 30), greatly diminished STAT3 activation (data not shown). The latter two mutations reduce Fes tyrosine kinase activity in vitro (30), which may account for their reduced efficiency of STAT3 activation.

To determine whether Fes exhibits a preference for STAT3, Fes was co-expressed with STAT5 in 293T cells. Cell lysates
Activation of STAT3 by Fes in 293T cells. Human 293T cells were transfected with STAT5, Fes, or a kinase-inactive Fes mutant (Fes-KE) either alone or in the combinations shown. Cytosolic extracts were prepared and tested for the presence of active STAT5 by gel-shift analysis with an MGFE probe. Control lanes include incubation of the probe in the absence of extract (MGFE) and gel-shift assays of untransfected cell extracts (293T). Duplicate gel-shift assays were conducted in the presence of a 100-fold excess of unlabeled MGFE to demonstrate probe specificity (COMP). The shifted STAT5-MGFE complex is denoted by the arrow.

STAT3 was expressed alone or in the presence of the kinase-inactive Fes mutant, K590E. Control blots show equivalent levels of STAT proteins, aliquots of the immunoprecipitates were also blotted with the STAT antibodies (center). To verify the expression of Fes and Fes-KE, the cytosolic extracts were immunoblotted with the anti-FLAG antibody, M2 (bottom).

**Fig. 2. Activation of STAT5 by Fes in 293T cells.** Human 293T cells were transfected with STAT5A, Fes, or a kinase-inactive Fes mutant (Fes-KE) either alone or in the combinations shown. Cytosolic extracts were prepared and tested for the presence of active STAT5 by gel-shift analysis with an MGFE probe. Control lanes include incubation of the probe in the absence of extract (MGFE) and gel-shift assays of untransfected cell extracts (293T). Duplicate gel-shift assays were conducted in the presence of a 100-fold excess of unlabeled MGFE to demonstrate probe specificity (COMP). The shifted STAT5-MGFE complex is denoted by the arrow.

**Fig. 3. Fes induces tyrosine phosphorylation of STAT3 and STAT5 in 293T cells.** Human 293T cells were transfected with STAT3, STAT5, Fes, or a kinase-inactive Fes mutant (Fes-KE) either alone or in the combinations shown. Untransfected cells were also included as an additional negative control (293T). Cytosolic extracts were prepared and incubated with antibodies to either STAT3 or STAT5. Immune complexes were precipitated with protein G-Sepharose and immunoblotted with antibodies to phosphotyrosine (PY20, top). To verify equivalent recovery of STAT proteins, aliquots of the immunoprecipitates were also blotted with the STAT antibodies (center). To verify the expression of Fes and Fes-KE, the cytosolic extracts were immunoblotted with the anti-FLAG antibody, M2 (bottom).
result observed in 293T cells (Fig. 1). The Fes-induced SIE-STAT3 complex was fully competed with a 100-fold excess of unlabeled SIE, indicative of binding specificity. In contrast, the kinase-inactive mutant of Fes produced no detectable increase in STAT3 activation above background. These results strongly suggest that Fes is capable of directly activating STAT3 without the requirement for a member of the JAK family or another intermediate tyrosine kinase.

The effect of Fes on STAT5 activation was also investigated in Sf-9 insect cells using the gel-shift assay and MGFE probe. As shown in Fig. 5, no detectable STAT5 DNA binding activity was observed in the presence of Fes. This result is in contrast to the tyrosine phosphorylation and activation of STAT5 by Fes observed in 293T cells (Figs. 2 and 3). Taken together, these results suggest that activation of STAT5 by Fes in 293T cells is indirect, possibly involving activation of an endogenous tyrosine kinase by Fes which in turn activates STAT5.

To control for the expression of Fes and STAT proteins in the Sf-9 cell experiments, immunoblots were performed on the cell extracts used for the gel-shift assays. As shown in Fig. 6, Fes and STAT proteins were all strongly expressed in the infected cells. In addition, immunoblots of the same extracts with an anti-phosphotyrosine antibody revealed potent tyrosine phosphorylation of STAT3 in the presence of Fes, consistent with the strong signal observed in the gel-shift assay (Fig. 4). Also visible in this experiment is autophosphorylated Fes. On the other hand, no tyrosine phosphorylation of STAT5 was detected, despite the presence of active Fes. This result is consistent with the negative gel-shift result with extracts from cells co-infected with Fes and STAT5 (Fig. 5).

An alternative explanation for the negative gel-shift result with Fes and STAT5 in Sf-9 cells is that the baculovirus-expressed STAT5 protein may be resistant to activation in the insect cell context. To rule out this possibility, STAT5 was co-expressed with the p210 form of Bcr-Abl, the constitutively activated protein-tyrosine kinase associated with chronic myelogenous leukemia (33). Several recent reports have shown that Bcr-Abl induces the activation of STAT5 as well as STATs 1 and 3 (10–12). Using the gel-shift assay with the MGFE probe, we observed that STAT5 was readily activated by Bcr-Abl in Sf-9 cells, whereas no detectable activation occurred with Fes (Fig. 7). This result indicates that baculovirus-expressed STAT5 can bind to DNA following phosphorylation by the appropriate tyrosine kinase. We also observed potent acti-
vation of STAT3 by Bcr-Abl in Sf-9 cells (Fig. 7). Our observation of Bcr-Abl-induced STAT activation in Sf-9 cells is consistent with previous work by Ilaria and Van Etten (12), which suggests that STATS are directly activated by Bcr-Abl and do not require JAKs or other endogenous kinases.

**DISCUSSION**

Data presented in this report demonstrate for the first time that STAT3 can be activated by the c-Fes tyrosine kinase. Our approach was to compare STAT activation in human versus insect cell backgrounds to address the issue of whether endogenous mammalian tyrosine kinases play a role in the activation mechanism. Our observations that Fes strongly activates STAT3 in both human and insect cells suggest that Fes is sufficient to activate STAT3 without a requirement for JAKs or other tyrosine kinase intermediates. However, the possible contribution of other tyrosine kinases to the activation mechanism in mammalian cells cannot be formally ruled out. These findings with STAT3 are consistent with our recent work showing that endogenous STAT3 activation occurs in fibroblasts transformed by v-fps, the retroviral homolog of c-Fes (13). Taken together, these data suggest that v-fps may directly induce endogenous STAT3 activation as well. In contrast to STAT3, however, we observed that Fes was unable to activate STAT5 in insect cells, despite some degree of activation in mammalian cells. These findings suggest that Fes activates STAT5 via an indirect mechanism, possibly via endogenous tyrosine kinases. Such a phenomenon has been reported previously for the activation of STATs by both v-abl and src kinases (8, 9).

Potent activation of STAT3 by Fes in transfected human cells suggests that such an event may also be induced by Fes in hematopoietic cells, an important site of Fes expression (14–16). Fes has been linked to the induction of myeloid differentiation in both normal and leukemic cell lines (21, 22, 34). Two recent studies have strongly implicated STAT3 as a key component of myeloid differentiation as well. Both of these studies utilized dominant-negative mutants of STAT3 (23, 24). These mutants were produced by substitution of the STAT3 tyrosine phosphorylation site with phenylalanine or by mutation of residues critical for DNA binding. When these mutants were introduced into M1 myeloid leukemia cells, the cells no longer responded to IL-6 with growth arrest and terminal differentiation. Interestingly, IL-6 is one of the cytokines reported to activate Fes (19). Taken together, these results suggest that Fes may contribute to the IL-6-mediated activation of STAT3 and the subsequent differentiation response.

Recent studies of GM-CSF-treated neutrophils also suggest a connection between Fes and STAT3 activation (35). In these experiments, GM-CSF was shown to induce the formation of a multiprotein complex consisting of Fes, Jak2, Stat1, Stat3, and the β-subunit of the GM-CSF receptor. However, this previous study did not address the possible contribution of Fes to STAT activation. Data presented here clearly demonstrate the tyrosine phosphorylation of STAT3 by Fes and induction of its DNA binding activity, strongly suggesting that this activation mechanism may be direct. The GM-CSF receptor may serve as a docking site to bring Fes and STAT3 into close proximity to allow the phosphorylation event to occur. Such an interaction may be initiated by Jak2, which could create binding sites for the SH2 domains of Fes and STAT3 on the receptor β-subunit. Further experiments will be required to address the possible contribution of Fes to STAT activation in response to cytokine treatment. However, a definitive demonstration that Fes is involved in endogenous STAT3 activation by GM-CSF, IL-6, or other cytokines may be difficult because of the multiple non-receptor tyrosine kinases activated in response to cytokine treatment.

Although our results in Sf-9 insect cells indicate that STAT5 is not a direct substrate for Fes, co-expression of these two proteins in human cells induced both STAT5 tyrosine phosphorylation and DNA binding activity. These results suggest that Fes may indirectly induce STAT5 activation under physiological conditions. Like STAT3, STAT5 may also contribute to hematopoietic differentiation in some cases. Recent studies have established a strong correlation between STAT5 activation and differentiation of myeloid leukemia cell lines (36). In these experiments, induction of monocyctic differentiation in human U937 cells with phorbol ester, retinoic acid, or 1α,25-dihydroxy-vitamin D₃ induced strong DNA binding activity of STAT5. Similar results were observed following chemically induced differentiation of HL-60 promyelocytic leukemia cells. In the same study, STAT5 activation was observed in primary cultures of chicken myeloblasts following differentiation to macrophages with chicken myelomonocytic growth factor. Another recent study has shown that induction of megakaryocytic differentiation by thrombopoietin correlates with activation of STAT5. This study also identified the cyclin-dependent kinase inhibitor p21WAF1/CIP1 as a possible target gene for STAT5 in the differentiation response (37). Further work will be required to determine whether Fes can stimulate similar STAT5 responses as part of a differentiation signaling pathway in hematopoietic cells.

**REFERENCES**

1. Ilie, J. N., Witthuhn, B. A., Quelle, F. W., Yamamoto, K., and Silvennoinen, O. (1995) *Annu. Rev. Immunol.* 13, 369–398.
2. Schindler, C., and Darnell, J. E., Jr. (1995) *Annu. Rev. Biochem.* 64, 621–651.
3. Ilie, J. N. (1996) *Cell* 84, 331–334.
4. Watanabe, S., and Araki, K. (1996) *Curr. Opin. Genet. Dev.* 6, 587–596.
5. Darnell, J. E., Jr. (1997) *Science* 277, 1630–1635.
6. Yu, C.-L., Meyer, D. J., Campbell, G. S., Lerner, A. C., Carter-Su, C., Schwartz, J., and Jove, R. (1995) *Science* 269, 1–3.
7. Cao, X. M., Tay, A., Guy, G. R., and Tan, Y. H. (1996) *Med. Cell. Biol.* 16, 1595–1603.
8. Campbell, G. S., Yu, C.-L., Jove, R., and Carter-Stu, C. (1997) *J. Biol. Chem.* 272, 2591–2594.
9. Danial, N. N., Pernis, A., and Rothman, P. B. (1995) *Science* 269, 1875–1877.
10. Shuai, K., Halpern, J., ten Hoeve, J., Rao, X. P., and Sawyer, C. L. (1996) *Oncogene* 13, 247–254.
11. Carless, F., Frank, D. A., and Griffin, J. D. (1996) *Exp. Med.* 183, 811–820.
12. Ilaria, R. L., Jr., and Van Etten, R. A. (1996) *J. Biol. Chem.* 271, 31704–31710.
13. Garcia, R., Yu, C.-L., Huhnl, A., Cattett, R., Nelson, K., Smithgall, T. E., Fujita, D. J., Ehrier, S., and Jove, R. (1998) *Cell Growth Diff.* 9, 1267–1276.
14. Smithgall, T. E., Yu, G., and Glazer, R. I. (1988) *J. Biol. Chem.* 263, 15500–15505.
15. Feldman, R. A., Gabrilove, J. L., Tam, J. P., Moore, M. A. S., and Hanafusa, H. (1985) *Proc. Natl. Acad. Sci. U. S. A.* 82, 2379–2383.
16. MacDonald, I., Levy, J., and Watson, P. (1985) *Mol. Cell. Biol.* 5, 2543–2553.
17. Hanazono, Y., Chiba, S., Sasaki, K., Mano, H., Yamauchi, Y., and Hirai, H. (1995) *Blood* 81, 3193–3196.
18. Hanazono, Y., Chiba, S., Sasaki, K., Mano, H., Miyajima, A., Araki, K., Yamauchi, Y., and Hirai, H. (1993) *EMBO J.* 12, 1641–1646.
19. Matsuda, T., Fukada, T., Takahashi-Tezuka, M., Okuyama, Y., Fujitani, Y., Hanazono, Y., Hirai, H., and Hirano, T. (1995) J. Biol. Chem. 270, 11037–11039
20. Izuhara, K., Feldman, R. A., Greer, P., and Harada, N. (1994) J. Biol. Chem. 269, 18623–18629
21. Yu, G., Smithgall, T. E., and Glazer, R. I. (1989) J. Biol. Chem. 264, 19276–19281
22. Carmier, J. F., and Samarut, J. (1986) Cell 44, 159–165
23. Nakajima, K., Yamanaka, Y., Nakae, K., Kojima, H., Ichiba, M., Kiuchi, N., Kataoka, T., Fukada, T., Hibi, M., and Hirano, T. (1996) EMBO J. 15, 3651–3658
24. Minami, M., Inoue, M., Wei, S., Takeda, K., Matsumoto, M., Kishimoto, T., and Akira, S. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 1963–1966
25. Pear, W. S., Nolan, G. P., Scott, M. L., and Baltimore, D. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 8392–8396
26. Rogers, J. A., Read, R. D., Li, J., Peters, K. L., and Smithgall, T. E. (1996) J. Biol. Chem. 271, 17519–17525
27. Pallard, C., Gouilleux, F., Charon, M., Groner, B., Gisselbrecht, S., and Dusanter-Fourt, I. (1995) J. Biol. Chem. 270, 15942–15945
28. Nelson, K. L., Becker, N. A., Pahwa, G. S., Hollingsworth, M. A., and Maher, L. J., III (1996) J. Biol. Chem. 271, 18061–18067
29. Yamanaka, Y., Nakajima, K., Fukada, T., Hibi, M., and Hirano, T. (1996) EMBO J. 15, 1557–1565
30. Hjermstad, S., Peters, K. L., Briggs, S. D., Glazer, R. I., and Smithgall, T. E. (1993) Oncogene 8, 2283–2292
31. Maru, Y., Peters, K. L., Afar, D. E. H., Shibuya, M., Witte, O. N., and Smithgall, T. E. (1995) Mol. Cell. Biol. 15, 835–842
32. Li, J., and Smithgall, T. E. (1996) J. Biol. Chem. 271, 32930–32936
33. Sawyer, C. L. (1992) Cancer Surv. 15, 37–51
34. Manfredini, R., Grande, A., Tagliafico, E., Barbieri, D., Zucchini, P., Citro, G., Zupi, G., Franceschi, C., Torelli, U., and Ferrari, S. (1995) J. Exp. Med. 178, 381–389
35. Brizzi, M. F., Aronica, M. G., Rosso, A., Bagnara, G. P., Yarden, Y., and Pegoraro, L. (1996) J. Biol. Chem. 271, 3562–3567
36. Wolfman, I., Mellitzer, G., Klessinger, M., Buchhart, D., Meinke, A., Beug, H., and Decker, T. (1997) J. Immunol. 159, 877–886
37. Matsumura, I., Ishikawa, J., Nakajima, K., Oritani, K., Tomiyama, Y., Miyagawa, J. I., Kato, T., Miyazaki, H., Matsuzawa, Y., and Kanakura, Y. (1997) Mol. Cell. Biol. 17, 2933–2943
Activation of STAT3 by the c-Fes Protein-tyrosine Kinase
Kristie L. Nelson, Jim A. Rogers, Tammy L. Bowman, Richard Jove and Thomas E.
Smithgall

J. Biol. Chem. 1998, 273:7072-7077.
doi: 10.1074/jbc.273.12.7072

Access the most updated version of this article at http://www.jbc.org/content/273/12/7072

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 37 references, 26 of which can be accessed free at
http://www.jbc.org/content/273/12/7072.full.html#ref-list-1