Proc. Natl. Acad. Sci. USA
Vol. 93, pp. 11681–11686, October 1996
Cell Biology

The cytokine-activated tyrosine kinase JAK2 activates Raf-1 in a p21^{ras}-dependent manner

(signal transduction/baculovirus/mammalian cells/oncogenes)

KAI XIA*†, NISHIT K. MUKHOPADHYAY*, ROGER C. INHORN*, DWAYNE L. BARBER*, PAUL E. ROSE*, ROBERT S. LEE*, RADHA P. NARSIMHAN*, ALAN D. D'ANDREA*, JAMES D. GRIFFIN*, AND THOMAS M. ROBERTS*‡§

*Dana–Farber Cancer Institute and Harvard–MIT Division of Health Sciences and Technology and ‡Department of Pathology, Harvard Medical School, Boston, MA 02115

Communicated by David M. Livingston, Dana–Farber Cancer Institute, Boston, MA, July 31, 1996 (received for review April 29, 1996)

ABSTRACT **JAK2, a member of the Janus kinase superfamily was found to interact functionally with Raf-1, a central component of the ras/mitogen-activated protein kinase signal transduction pathway. Interferon-γ and several other cytokines that are known to activate JAK2 kinase were also found to stimulate Raf-1 kinase activity toward MEK-1 in mammalian cells. In the baculovirus coexpression system, Raf-1 was activated by JAK2 in the presence of p21^{ras}. Under these conditions, a ternary complex of p21^{ras}, JAK2, and Raf-1 was observed. In contrast, in the absence of p21^{ras}, coexpression of JAK2 and Raf-1 resulted in an overall decrease in the Raf-1 kinase activity. In addition, JAK2 phosphorylated Raf-1 at sites different from those phosphorylated by pp60$^{v\text{-}src}$. In mammalian cells treated with either erythropoietin or interferon-γ, a small fraction of Raf-1 coimmunoprecipitated with JAK2 in lysates of cells in which JAK2 was activated as judged by its state of tyrosine phosphorylation. Taken together, these data suggest that JAK2 and p21^{ras} cooperate to activate Raf-1.**

The serine/threonine kinase Raf-1 is activated by numerous growth factors (1, 2) and is believed to play a central role in cell growth and differentiation. Binding of a growth factor to its tyrosine kinase receptor at the cell surface leads to p21^{ras} activation that in turn recruits Raf-1 to the plasma membrane (3, 4) through a direct interaction with the N-terminal region of Raf-1. At the cell membrane, Raf-1 is activated in a p21^{ras}-independent manner. Activated Raf-1 phosphorylates and activates MEK-1, leading to activation of the mitogen-activated protein (MAP) kinase cascade (5, 6). Unlike members of the tyrosine kinase receptor superfamily, the cytokine receptors have no intrinsic tyrosine kinase activity, but are dependent mainly upon the JAK family of tyrosine kinases with which they form a stable complex (7–10). Binding of a cytokine to its receptor causes the receptor to dimerize, thereby activating the associated JAK kinases through tyrosine phosphorylation. Activated JAK kinases in turn phosphorylate a number of important cellular substrates, chief among which are members of the STAT family of transcription factors (11–14). Previous studies have documented that tyrosine phosphorylation of STATs leads to their oligomerization and is essential for activation of their DNA binding activity (15, 16). However, recently it has become apparent that subsequent phosphorylation of STATs on serine/threonine residues is required for their full activation as transcription factors (17, 18). It has also been reported that JAK2, p21^{ras}, and Raf-1 are required for the activation of MAP kinases by growth hormone stimulation (19), and in the interferon (IFN) system, STAT activation requires functionally activated MAP kinases (20). This focuses particular interest on the molecular mechanisms by which cytokine receptor family members couple to Raf-1 and to the MAP kinase cascade. Before the discovery of either MEK-1 as a physiological substrate of Raf-1 or the linkage of JAK kinases with cytokine receptors, it was demonstrated that in hematopoietic cells, stimulation of either granulocyte/macrophage colony-stimulating factor (GM-CSF), erythropoietin (EPO), or interleukin 2 (IL-2) receptors resulted in Raf-1 hyperphosphorylation (21–23). This data prompted us to search for a functional link between Raf-1 and JAK kinases. Using both a baculovirus system and cytokine-stimulated mammalian cell lines, we found that Raf-1 is not only phosphorylated on tyrosine residues when JAK2 is active, but also is activated by JAK2 in the presence of p21^{ras}.

MATERIALS AND METHODS

Cytokines and Antibodies. Recombinant human EPO and IL-2 were from Boehringer Mannheim. IFN-γ and GM-CSF were from Endogen (Cambridge, MA). Polyclonal anti-JAK2 antibody was purchased from Upstate Biotechnology (Lake Placid, NY). Rabbit polyclonal anti-Raf-1 serum was raised against a peptide corresponding to the C-terminal 12 residues of Raf-1 (CTLTTSPRLPVF). Mouse monoclonal antibody specific for phosphotyrosine (4G10) was generated in our own laboratory. Rat (clone Y13-238) and mouse (clone F111-85) monoclonal antibodies recognizing p21^{ras} were purchased from Oncogene Science.

Cells and Cell Culture. *Spodoptera frugiperda* (Sf21) cells were grown either in suspension or as a monolayer culture in Grace's medium (GIBCO/BRL 350-1605AJ) supplemented with 10% fetal calf serum. Human HeLa cells were grown in DMEM (GIBCO/BRL) supplemented with 10% calf serum. CTLL-EPO receptor (EPOR) and BaF/3-GM-CSFR cell lines were grown in RPMI medium 1640 supplemented with 10% fetal calf serum.

Preparation of Baculovirus-Infected Sf21 Cell Lysates and Cytokine-Stimulated Cell Lysates. Sf21 cells (2×10^6) were infected with the desired recombinant baculovirus or with combinations of the different recombinant baculoviruses in appropriate ratios. At 48 h postinfection, cell lysates were prepared in Nonidet P-40 lysis buffer [20 mM Tris, pH 8.0/137 mM NaCl/1 mM $MgCl_2$/10% (vol/vol) glycerol/1% (vol/vol) Nonidet P-40] supplemented with phenylmethylsulfonyl fluoride (1 mM), aprotinin (0.15 unit/ml), DTT (1 mM), and sodium orthovanadate (1 mM). HeLa cells (5×10^6) were stimulated with human recombinant IFN-γ (15 ng/ml) for 10 min at 37°C. Appropriately starved CTLL-EPOR cells (2×10^7) were stimulated with 50 units/ml of either IL-2 or EPO

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: EPO, erythropoietin; EPOR, erythropoietin receptor; IL-2, interleukin 2; IFN-γ, interferon-γ; MAP, mitogen-activated protein; GM-CSF, granulocyte/macrophage colony-stimulating factor.

§To whom reprint requests should be addressed.

for 10 min at 37°C. Cell lysates were prepared essentially as described above.

Immunoprecipitation and Western Blot Analysis. For $p21^{ras}$ immunoprecipitation, the rat anti-$p21^{ras}$ monoclonal antibody (Y13-238) was incubated with rabbit anti-rat IgG coupled to protein A-Sepharose beads and the appropriate amount of lysate for at least 6 h at 4°C. For JAK2 and Raf-1 immunoprecipitation, the rabbit anti-Raf-1 antibody or anti-JAK2 antibody was incubated directly with protein A-Sepharose beads and the appropriate amount of lysate for at least 4 h at 4°C. The beads bound to the specific antibody were washed three times with modified Nonidet P-40 lysis buffer containing 0.2% Nonidet P-40 and once with kinase buffer (25 mM Hepes, pH 7.4/1 mM DTT/10 mM $MgCl_2$/10 mM $MnCl_2$). The samples were then resolved by an 8% SDS/PAGE and transferred onto nitrocellulose paper. The blots were probed with the desired antibody and developed by enhanced chemiluminescence (ECL, Amersham).

***In Vitro* Kinase Assays.** The Raf-1 immunoprecipitates were prepared as above and the beads bound to anti-Raf antibody were washed twice with Nonidet P-40 lysis buffer, once with 0.5 M $LiCl_2$, and once with kinase buffer. The washed immunoprecipitates were then incubated in 40 μl of kinase buffer containing 15 μM nonradioactive ATP, 10 μCi (1 Ci = 37 GBq) of [γ-^{32}P]ATP (3000 Ci/mmol), and 0.1 μg 5′-p-fluorosulfonyl-benzoyladenosine-treated MEK-1 at room temperature for 30 min. The assays were terminated by addition of Laemmli sample buffer and resolved by SDS/PAGE. The phosphorylation of MEK-1 was quantitated by PhosphorImager and the amount of Raf-1 protein in each lane was either quantitated by a Western blot and FluorImager or qualitatively by ECL as previously described (24).

RESULTS

Activation of Raf-1 Kinase in Response to Cytokine Treatment as well as in Coexpression of JAK2 and $p21^{ras}$. Interferons and cytokines, which are known to activate JAK kinases, were first examined for their ability to activate the kinase activity of Raf-1 toward MEK-1. Raf-1 immunoprecipitates were made from IFN-γ-treated HeLa cells as well as from untreated controls. As shown in Fig. 1*A*, the stimulation of HeLa cells with IFN-γ increased the kinase activity of Raf-1 immunoprecipitates toward MEK-1. Similar results were obtained for IFN-γ treatment of NIH 3T3 cells and IL-2 and EPO treatment of CTLL-EPOR cells (data not shown).

To assess a functional link between the activation of Raf-1 and JAK2 kinases, we used the baculovirus expression system. This system allows a detailed study of functional interactions between Raf-1, $p21^{ras}$, and $pp60^{v\text{-}src}$ family kinases (25). In the baculovirus system, two signals must synergize for the efficient activation of Raf-1 autophosphorylation and substrate-directed kinase activities. One signal is supplied by $p21^{ras}$ that serves to localize Raf-1 to the membrane. The second signal is supplied by tyrosine kinases, such as $pp60^{v\text{-}src}$, and is independent of $p21^{ras}$ (26). Either signal alone gives a modest activation of Raf-1. Since many cytokines activate both Raf-1 and JAK2, we initially tested the ability of JAK2 to activate Raf-1. Raf-1 kinase activity was measured using highly purified inactive MEK-1 as a substrate in Raf-1 immunoprecipitates from insect cells infected with various recombinant baculoviruses as indicated. The kinase activity of Raf-1 from a single infection was normalized to 1 (Fig. 1*B*). Coexpression of Raf-1 with both $p21^{ras}$ and JAK2 resulted in higher levels of both Raf-1 autokinase and transkinase activity than were observed with Raf-1 alone or with Raf-1 and $p21^{ras}$ in combination. The absolute levels of Raf-1 activation by $p21^{ras}$ and JAK2 were not as high as those observed for $p21^{ras}$ and $pp60^{v\text{-}src}$. This may be due to the difference in the sites of phosphorylation on Raf-1 by the two tyrosine kinases (see below) or to the relatively low

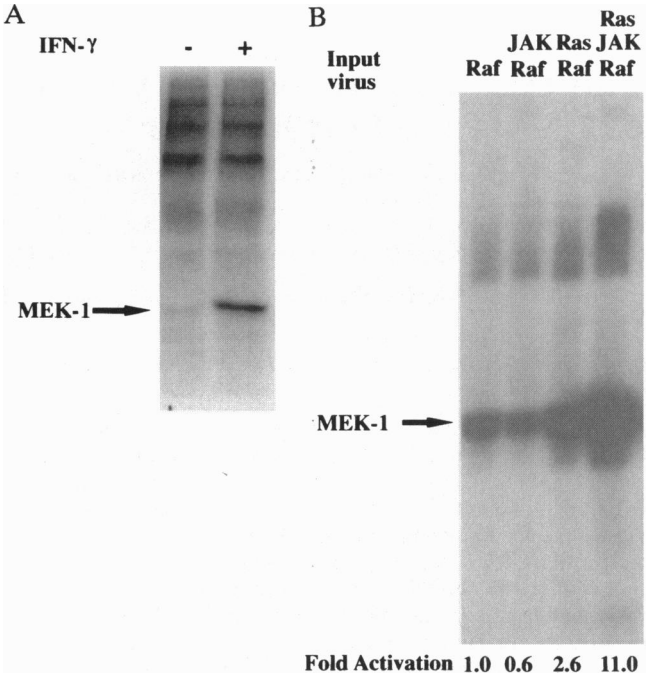

FIG. 1. (*A*) Activation of Raf-1 activity in IFN-γ-treated HeLa cells. The exponentially growing HeLa cells were unstimulated (−) or stimulated (+) with IFN-γ (15 ng/ml) for 10 min. Raf-1 immune complexes were prepared and subjected to an *in vitro* kinase assay using the purified, inactivated MEK-1 as a substrate. (*B*) Specific activation of Raf-1 kinase by JAK2 in the presence of $p21^{ras}$. Anti-Raf immunoprecipitates were prepared from Sf21 cells infected with Raf-1 alone or in combination with baculoviruses encoding JAK2, or $p21^{ras}$ or both. Extensively washed immunoprecipitates were subjected to an *in vitro* kinase assay using purified MEK-1 as a substrate. The resulting phosphorylated products were resolved on an 8% SDS/PAGE and transferred onto nitrocellulose paper. The phosphorylated MEK-1 and the amounts of Raf-1 used in each lane were quantitated by PhosphorImager and FluorImager, respectively. Data were normalized accordingly and the activation fold was calculated in comparison with Raf-1 activity toward MEK-1.

level of activity of JAK2 in the system. Interestingly, coexpression of Raf-1 and JAK2 consistently showed a slight decrease in Raf-1 kinase activity. This contrasts with previous results with $pp60^{v\text{-}src}$, which activates Raf-1 even in the absence of increased $p21^{ras}$ expression in this system.

Interaction of Raf-1 and $p21^{ras}$ with JAK2. In the course of our kinase assays, we observed a potential interaction between Raf-1 and JAK2. To examine this interaction further, we performed Western blot analysis of Raf-1 immunoprecipitates from Sf21 cells infected singly with baculovirus encoding Raf-1 or doubly with Raf-1 and JAK2. JAK2 was easily detectable in Raf-1 immunoprecipitates (Fig. 2*A*). The binding specificity was confirmed by the addition of the synthetic peptide corresponding to the 12 amino acids from the C terminus of Raf-1 against which the antibody was raised (Fig. 2*A*, lanes 1 and 3). It was also possible to do the reciprocal experiments in which Raf-1 could be blotted in JAK2 immunoprecipitates (data not shown). Interestingly, when $p21^{ras}$ immune complexes were blotted with a mixture of Raf-1 and JAK2 specific antibodies, a tripartite complex formation of Raf-1, $p21^{ras}$, and JAK2 was observed (Fig. 2*B*). Control experiments revealed that neither the wild-type $p21^{ras}$ nor the dominant negative rasN17 binds to JAK2 in the absence of Raf-1 (Fig. 2*C*).

Tyrosine Phosphorylation of Raf-1 in Coexpression with JAK2. Raf-1 is known to be phosphorylated on tyrosine residues in the baculovirus system when coexpressed with src family kinases or certain receptor tyrosine kinases. The sites of this phosphorylation have been mapped to tyrosines 340 and

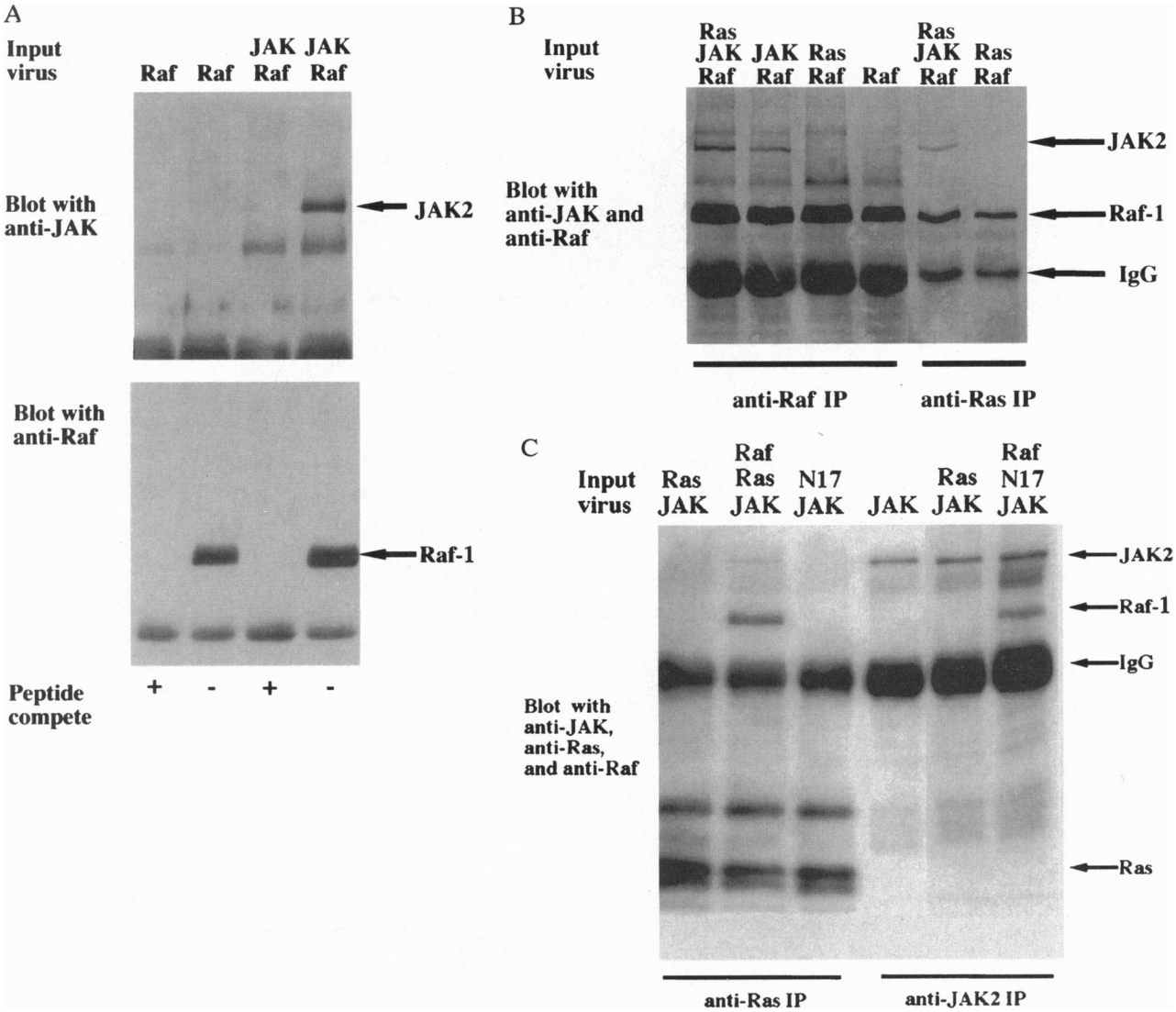

FIG. 2. Specific interactions between JAK2 and Raf-1 in Sf21 cells. (*A*) Insect cells (Sf21) were either infected with recombinant baculovirus encoding Raf-1 or in combination with baculovirus encoding JAK2. Anti-Raf-1 immunoprecipitates were prepared in the presence (+) or absence (−) of the synthetic Raf-1 peptide (10 nM), and analyzed by SDS/PAGE and immunoblotted with anti-JAK2 antibodies (*Upper*) or anti Raf-1 antibodies (*Lower*). The immunoblots were developed using ECL. (*B*) A tripartite complex formation among JAK2, $p21^{ras}$, and Raf-1. Raf-1 or $p21^{ras}$ immune complexes were prepared with rabbit anti-Raf-1 or rat anti-$p21^{ras}$ monoclonal antibody (Y13-238) from Sf21 cells infected with baculoviruses encoding various proteins as indicated. The immunoblot was probed with a mixture of antibodies against Raf-1 and JAK2. (*C*) The immunoprecipitates were prepared as above, and the blot was probed with a mixture of antibodies against Raf-1, JAK2, and $p21^{ras}$ (F111-85).

341 (27). Mutation of these sites renders Raf-1 insensitive to $pp60^{v\text{-}src}$. Upon coexpression with JAK2, Raf-1 is also phosphorylated on tyrosine although to a lower level than is seen for $pp60^{v\text{-}src}$ (Fig. 3*A Left*). The mutant allele of Raf-1 in which tyrosines 340 and 341 have both been changed to phenylalanines is still phosphorylated on tyrosine residues by JAK2 but not by $pp60^{v\text{-}src}$. This indicates that Raf-1 is phosphorylated on sites other than 340/341. To test the functional importance of these additional phosphorylations, we performed a kinase assay on the Raf-1 mutant 340/341. As shown in Fig. 3*B*, JAK2 was able to activate 340/341 in the presence of $p21^{ras}$ but to a much lower extent than is seen with wild-type Raf-1. This indicates that the sites at 340/341 are indeed phosphorylated by JAK2 and that this phosphorylation represents a major component of the activation process. However, it is worth noting that $pp60^{v\text{-}src}$ also activates the 340/341 mutant protein to a similar degree in the presence of $p21^{ras}$; therefore, it is possible that this residual activation by both $pp60^{v\text{-}src}$ and JAK2 may be due to a different mechanism than tyrosine phosphorylation. Interestingly, the level of JAK2 tyrosine phosphorylation is markedly decreased upon coexpression with Raf-1 (Fig. 3*A Right*).

Interaction of Raf-1 with JAK2 in Mammalian Cells. We also examined the physical interactions of Raf-1 with JAK2 in mammalian cells responding to IFN-γ, EPO, and GM-CSF. Receptors for each of these ligands interact with and activate JAK2. Fig. 4*A* shows the results from a T-cell line (CTLL-EPOR) that naturally expresses IL-2 receptor and has been engineered to express EPOR. Upon stimulation with IL-2, JAK1 and JAK3, but not JAK2, are activated, whereas upon stimulation with EPO, only JAK2 is activated (30, 31). Cells were treated with the relevant ligand, and Raf-1 immunoprecipitates were prepared and blotted with anti-JAK2 antibodies (Fig. 4*A Upper*). In unstimulated or IL-2-stimulated CTLL-EPOR cells, little binding of Raf-1 and JAK2 was observed. However, in EPO-stimulated cells, JAK2 appears to be associated with Raf-1. The blot was stripped and reprobed with anti-phosphotyrosine antibody (Fig. 4*A Lower*). As expected, JAK2 was markedly phosphorylated on tyrosine in EPO-stimulated cells (lanes 3 and 6). Interestingly, a band of the

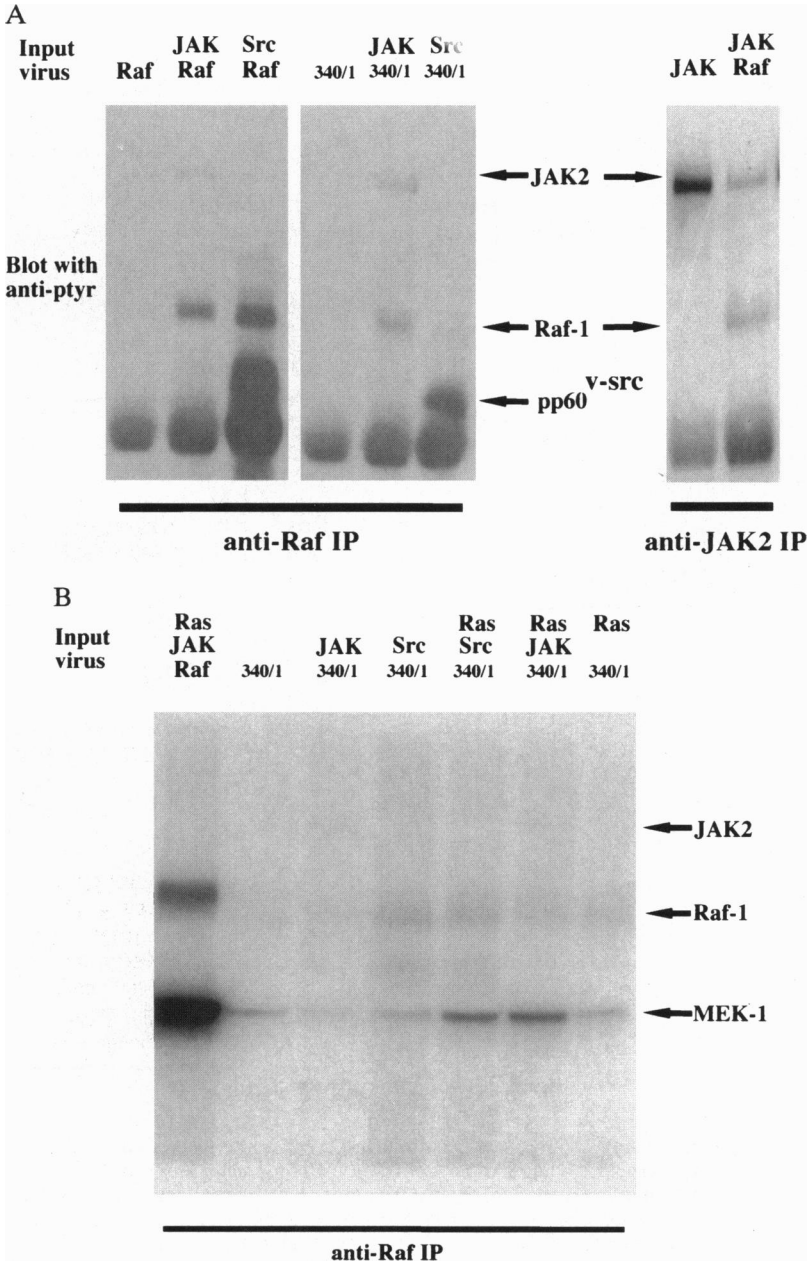

FIG. 3. (*A Left*) Differential tyrosine phosphorylation of Raf-1 by JAK2 and pp60$^{v\text{-}Src}$. Anti-Raf immunoprecipitates were prepared from Sf21 cells singly infected with baculoviruses encoding either Raf-1 or a mutant Raf-340/341 or doubly infected with baculoviruses encoding Raf-1 or Raf-340/341 in combination with either JAK2 or pp60$^{v\text{-}src}$. The immunoblot was probed with anti-phosphotyrosine antibody and analyzed using ECL. (*A Right*) Effect of Raf-1 on tyrosine phosphorylation of JAK2. Anti-JAK2 immunoprecipitates were prepared from Sf21 cells singly infected with baculovirus encoding JAK2 or doubly infected with baculoviruses encoding Raf-1 and JAK2. The immunoblot was probed with anti-phosphotyrosine antibody and analyzed using ECL. (*B*) Activation of Raf-340/341 mutant protein by JAK2 or pp60$^{v\text{-}src}$ in cooperation with p21^{ras}. Anti-Raf immune complexes were prepared from Sf21 cells infected with Raf-340/341 alone or in combination with baculoviruses encoding JAK2 or pp60$^{v\text{-}src}$ and p21^{ras}. An *in vitro* kinase assay was performed essentially as described in Fig. 1*B*. The resulting phosphorylated products were resolved on an SDS/8% polyacrylamide gel and transferred onto nitrocellulose paper. The phosphorylated MEK-1 was revealed by exposing the blot to Kodak X-AR film and the amounts of Raf-340/341 used in each lane were indicated by specific antibody reaction and ECL (data not shown).

exact mobility of JAK2 is seen in the antiphosphotyrosine blots of Raf-1 immunoprecipitates from EPO-treated cells. These data are consistent with an association between Raf-1 and activated forms of JAK2. We also examined the interaction of Raf-1 and JAK2 in Pro-B cells (Ba/F3) engineered to express GM-CSF receptor. Raf-1 and JAK2 coimmunoprecipitated in the cases in which JAK2 was tyrosine phosphorylated (data not shown). Similar results were obtained for HeLa cells treated with IFN-γ (Fig. 4*B*). However, we were unable to detect Raf-1 in JAK2 immunoprecipitates from EPO or IFN-γ treated cells in a reproducible fashion. This may be due to the low levels of the Raf-1/JAK2 complex or to the relatively limited ability of the anti-JAK2 serum to recognize JAK2 in complex with Raf-1.

DISCUSSION

Our data provide a key molecular link between two signaling pathways. It has become clear that in addition to eliciting the tyrosine phosphorylation of STATs, cytokine receptors often

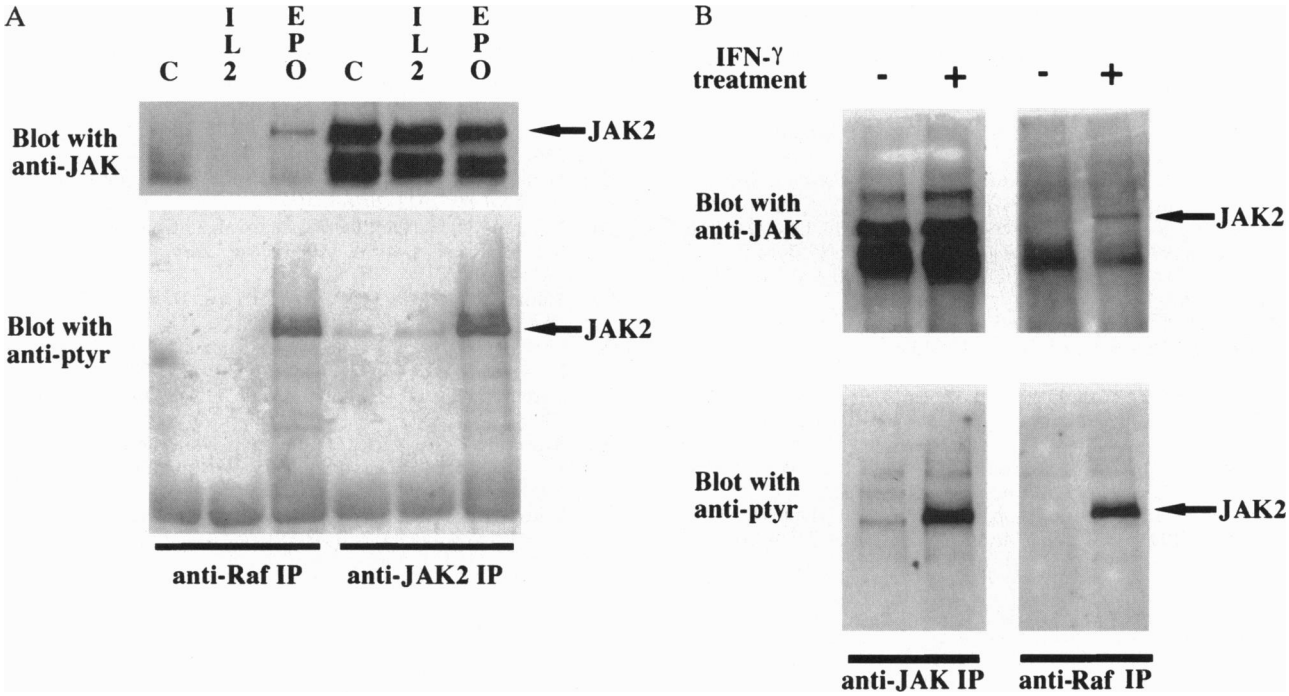

FIG. 4. (*A*) Coimmunoprecipitation of JAK2 with Raf-1 in CTLL-EPOR cells. CTLL cells engineered to express EPOR (28, 29) were either unstimulated (lanes C) or stimulated with IL-2 (50 units/ml) or EPO (50 units/ml). The cell extracts were incubated with anti-Raf antibody (the first three lanes) or anti-JAK2 antibody (the last three lanes). The immunoprecipitates were analyzed by SDS/8% polyacrylamide gel and immunoblotted with anti-JAK2 antibodies (*Upper*) or with antiphosphotyrosine antibody (*Lower*). The immunoblots were developed using ECL. (*B*) Coimmunoprecipitation of JAK2 with Raf-1 in HeLa cells. HeLa cells were either untreated or treated with recombinant IFN-γ as described in Fig. 1*A*. The preparation and analysis of Raf-immunoprecipitates were basically the same as indicated in Fig. 4*A*.

activate MAP kinases. Here we demonstrated that not only can JAK2 activate Raf-1 in the presence of $p21^{ras}$, but also that it may form a complex with its target. Based on our data, we propose the following model. Cytokine receptors, when stimulated, activate JAK2 kinase, which in turn binds Raf-1. Notably, formation of this Raf-1/JAK2 complex alone is not sufficient to activate Raf-1. Instead Raf-1 is sequestered until the $p21^{ras}$ protein, activated by the same or another cytokine receptor, enters the complex. Ras entrance triggers the activation of Raf-1 and may initiate the release of the Raf/Ras complex from the cytokine receptor. A key question that remains to be answered concerns the mechanism by which JAK2 contributes to the activation of Raf-1. In the case of $pp60^{v\text{-}src}$ activation of Raf-1 in baculovirus, it appears that tyrosine phosphorylation of Raf-1 on tyrosines 340/341 is essential. While tyrosine phosphorylation of Raf-1 by JAK2 occurs at sites in addition to 340/341 in the baculovirus system, our data show that mutation of 340/341 is sufficient to block most, but not all, of the activation by JAK2 and $p21^{ras}$. We are now in the process of mapping the new sites to facilitate genetic analysis of the problem. Meanwhile, we note that tyrosine phosphorylation of Raf-1 is detectable in EPO-, GM-CSF-, or IFN-γ-stimulated mammalian cells (unpublished data). This raises the possibility that tyrosine phosphorylation may play a role in Raf-1 activation in response to cytokine stimulation of cells.

The physiological relevance of the interaction between Raf-1 and JAK2 will also require further study. This type of binding has been previously observed for Raf-1 and the platelet-derived growth factor receptor. It can be argued that it is mechanistically favorable to bring the activating tyrosine kinase together with its downstream target. However, the role of $p21^{ras}$ in bringing Raf-1 to the membrane must not be neglected. While in our model we envision an interaction between Raf-1 and JAK2 preceeding the arrival of $p21^{ras}$, it is quite possible that under physiological conditions $p21^{ras}$ first brings Raf-1 to the membrane, and only then does Raf-1 interact with the activated JAK kinase. In mammalian cells we see very low levels of the JAK2/Raf-1 complex. This might suggest that the complex is not a major element in signaling under physiological conditions, but it should be noted that even the well established interaction between $p21^{ras}$ and Raf-1 is difficult to detect at physiological levels of expression for the interacting proteins.

We thank Debbie Morrison for the recombinant baculovirus 340/341, Ibrahim A. Aksoy and H. Toni Jun for preparation and critical reading of the manuscript, and Ben Neel for helpful discussions. R.C.I. is a fellow of Leukemia Society of America. This work was supported by the National Institutes of Health Grants CA43803 (T.M.R.), DK43889 (A.D.D.), and CA36167 (J.D.G.). In compliance with Harvard Medical School guidelines on possible conflict of interest, we disclose that one of the authors (T.M.R.) has consulting relationships with Upstate Biotechnology and Sandoz Pharmaceuticals, Inc.

1. Roberts, T. M. (1992) *Nature (London)* **360,** 534–535.
2. Marshall, C. J. (1995) *Cell* **80,** 179–185.
3. Leevers, S. J., Paterson, H. J. & Marshall, C. J. (1994) *Nature (London)* **369,** 411–414.
4. Stokoe, D., Macdonald, S. G., Cadwallader, K., Symons, M. & Hancock, J. F. (1994) *Science* **264,** 1463–1467.
5. Alessi, D. R., Saito, Y., Campbell, D. J., Cohen, P., Sithanandam, G., Rapp, U., Ashworth, A., Marshall, C. J. & Cowley, S. (1994) *EMBO J.* **13,** 1610–1619.
6. Zheng, C. F. & Guan, K. L. (1994) *EMBO J.* **13,** 1123–1131.
7. Velazquez, L., Fellous, M., Stark, G. R. & Pellegrini, S. (1992) *Cell* **70,** 313–322.
8. Hunter, T. (1993) *Nature (London)* **366,** 114–116.
9. Muller, M., Briscoe, J., Laxton, C., Guschin, D., Ziemiecki, M., Silvennoinen, O., Harpur, A. G., Barbieri, G., Witthuhn, B. A., Schindler, C., Pellegrini, S., Wilks, A. F., Ihle, J. N., Stark, G. R. & Kerr, I. M. (1993) *Nature (London)* **366,** 129–135.
10. Watling, D., Guschin, D., Muller, M., Silvennoinen, O., Witthuhn, B. A., Quelle, W. F., Rogers, N. C., Schindler, C., Stark,

G. R., Ihle, J. N. & Kerr, I. M. (1993) *Nature (London)* **366,** 166–170.
11. Shuai, K., Ziemiecki, A., Wilks, A. F., Harper, A. G., Sadowski, H. B., Gilman, M. Z. & Darnell, J. E., Jr. (1993) *Nature (London)* **366,** 580–583.
12. Schindler, C., Shuai, K., Prezioso, V. R. & Darnell, J. E., Jr. (1992) *Science* **257,** 809–813.
13. Shuai, K., Schindler, C., Prezioso, V. R. & Darnell, J. E., Jr. (1992) *Science* **258,** 1808–1812.
14. Fu, X. Y., Kessler, D. S., Veals, S. A., Levy, D. E. & Darnell, J. E., Jr. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 8553–8559.
15. Shuai, K., Stark, G. R., Kerr, I. M. & Darnell, J. E., Jr. (1993) *Science* **261,** 1744–1746.
16. Ihle, J. N. & Kerr, I. M. (1995) *Trends Genet.* **11,** 69–74.
17. Zhang, X., Blenis, J., Li, H.-C., Schindler, C. & Chen-Kiang, S. (1995) *Science* **267,** 1990–1994.
18. Wen, Z., Zhong, Z. & Darnell, J. E., Jr. (1995) *Cell* **82,** 241–250.
19. Winston, L. A. & Hunter, T. (1995) *J. Biol. Chem.* **270,** 30837–30840.
20. David, M., Petricoin, E., III, Benjamin, C., Pine, R., Weber, M. J. & Larner, A. C. (1995) *Science* **269,** 1721–1723.
21. Turner, B., Rapp, U. R., App, H., Greene, M., Dobashi, K. & Reed, J. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 1227–1231.
22. Carroll, M. P., Spivak, J. L., McMahon, M., Weich, N., Rapp, U, R. & May, W, S. (1991) *Mol. Cell. Biol.* **266,** 14964–14969.
23. Zmuidzinas, A., Mamon, H., Roberts, T. M. & Smith, K. A. (1991) *Mol. Cell. Biol.* **11,** 2794–2803.
24. Fu, H., Xia, K., Pallas, D. C., Cui, C., Conroy, K., Narsimhan, R. P., Mamon, H., Collier, R. J. & Roberts, T. M. (1994) *Science* **266,** 126–129.
25. Williams, N. G., Roberts, T. M. & Li, P. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 2922–2926.
26. Williams, N. G., Paradis, H., Agarwal, S., Charest, D. L., Pelech, S. L. & Roberts, T. M. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 5772–5776.
27. Fabian, J. R., Daar, I. O. & Morrison, D. K. (1993) *Mol. Cell. Biol.* **13,** 7170–7179.
28. Showers, M. O., Moreau, J. F., Linnekin, D., Druker, B. & D'Andrea, A. D. (1992) *Blood* **80,** 3070–3078.
29. Barber, D, L. & D'Andrea, A. D. (1994) *Mol. Cell. Biol.* **14,** 6506–6514.
30. Miyazaki, T., Kawahara, A., Fujii. H., Nakagawa, X., Minami, Y., Liu, Z., Oishi, I., Silvennoinen, O., Witthuhn, B. A., Ihle, J. N. & Taniguchi, T. (1994) *Science* **266,** 1045–1047.
31. Witthuhn, B. A., Quelle, F. W., Silvennoinen, O., Yi, T., Tang, B., Miura, O. & Ihle, J. N. (1993) *Cell* **74,** 227–236.