Thrombopoietin Signal Transduction Requires Functional JAK2, Not TYK2*

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The Janus family of tyrosine kinases (JAKs) plays a critical role in signal transduction by members of the cytokine receptor superfamily. In response to ligand-receptor interaction, these nonreceptor tyrosine kinases are rapidly phosphorylated and activated, triggering tyrosine phosphorylation and activation of downstream signaling intermediates. Upon binding to its receptor, the product of the proto-oncogene c-mpl, thrombopoietin (TPO) activates both JAK2 and TYK2 in multiple cell lines as well as megakaryocytes and platelets. To study whether one or both of these kinases are essential for TPO signal transduction, we engineered a parental human sarcoma cell line (2C4) as well as sarcoma cell lines that are deficient in JAK2 expression (γ2A) or TYK2 expression (U1A) to express the wild-type Mpl receptor. The ability of TPO to induce tyrosine phosphorylation of Mpl and multiple intracellular substrates in each cell line was then examined. Our results demonstrate that JAK2-deficient cells (γ2A-Mpl) are unable to initiate TPO-mediated signaling. In contrast, cells that are TYK2-deficient (U1A-Mpl) are able to induce tyrosine phosphorylation of Mpl, JAK2, STAT3, and Shc as efficiently as parental cells (2C4-Mpl). These data indicate that JAK2 is an essential component of Mpl signaling and that, in the absence of JAK2, TYK2 is incapable of initiating TPO-induced tyrosine phosphorylation.

Tyrosine phosphorylation and activation of the Janus tyrosine kinases (JAK)1 is an essential element of signal transduction by all members of the hematopoietic cytokine receptor family (reviewed in Refs. 1 and 2). There are four members of the Janus kinase family (JAK1, JAK2, JAK3, and TYK2), cytoplasmic proteins that associate with the intracellular portion of cytokine receptors via conserved box 1 and box 2 motifs in the membrane-proximal portion of the receptor. The box 1 motif is defined by two characteristically spaced prolines (XXP) located within 30 residues of the transmembrane domain (3). Box 2 is usually located 35–60 residues downstream of the transmembrane domain, and contains 12–18 residues, rich in glutamic acid and serine (3). It is likely that the specificity of JAK-receptor interactions is mediated through these regions, although no definitive JAK binding motifs have been recognized. Ligand binding leads to receptor multimerization and permits trans-phosphorylation and activation of the associated kinases. The utilization of a specific JAK or pair of JAKs is characteristic of each receptor (reviewed in Refs. 1, 4, and 5).

Thrombopoietin (TPO), the primary regulator of platelet production (6), is structurally and functionally related to a large family of hormones, cytokines, and interleukins. Upon binding to its receptor, Mpl, TPO is believed to induce receptor homomimerization. This hypothesis is supported by the absence of interaction with the known common signaling subunits (gp130, IL-2 receptor-γ, and the common β-chain of the GM-CSF, IL-3, and IL-5 receptors) as well as reports that Mpl homodimerization is sufficient for signaling (7–11). In general, homodimeric receptors utilize a single JAK (e.g. erythropoietin and prolactin receptors utilize JAK2), whereas heterodimeric (or heterotrimetric) receptors require two distinct JAKs (e.g. interferon-α uses JAK1 and TYK2, interferon-γ uses JAK1 and JAK2) (reviewed in Ref. 12). Thus, it was surprising when several reports demonstrated that TPO induces phosphorylation of two distinct Janus family members, JAK2 and TYK2. This has been observed both in cell lines engineered to express the Mpl receptor (13, 14) as well as human platelets (15), which likely represent a more physiologically relevant environment. Others have shown that, of the four Janus kinases, only JAK2 is tyrosine-phosphorylated in response to TPO (16, 17). In our previous studies, we reported that there was a difference in JAK phosphorylation between cell lines and purified, primary megakaryocytes. Whereas cell lines, which proliferate in response to TPO, demonstrated abundant JAK2 and TYK2 phosphorylation after TPO stimulation, mature megakaryocytes contained primarily JAK2 phosphorylation; TYK2 tyrosine phosphorylation was barely detectable (18). This raised several important questions about the earliest events during TPO signaling. Are JAK2 and TYK2 both essential for Mpl signaling? What would be the consequence if JAK2 or TYK2 were absent during TPO/Mpl signaling?

To address these questions, we utilized human sarcoma cells that were originally selected on the basis of their failure to transduce an interferon-specific signal. These cells were found to lack normal expression of either JAK2 (γ2A) or TYK2 (U1A) and interfere with interferon-γ or interferon-α signaling, respectively (12). These cells have previously been used to demonstrate that although IL-6 stimulation induces tyrosine phosphorylation of three distinct Janus kinases (JAK1, JAK2, and TYK2), JAK1 is most important for activation of downstream signaling molecules (19). Based on this strategy, we engineered each of these cell lines and the corresponding parental cells to express the full-length murine Mpl receptor and then assessed the ability of TPO to initiate signal transduction. Our results demonstrate that JAK2, but not TYK2, is required for tyrosine phosphorylation of three distinct Janus kinases.
phosphorylation of Mpl, STAT3, and Shc. This suggests that TYK2 phosphorylation, although readily detected in various cell lines, is not an essential step in TPO signaling. Dependence on a single Janus kinase is consistent with our understanding of homodimeric receptors.

**Experimental Procedures**

**Reagents**—Monoclonal phosphotyrosine antibody (4G10) and polyclonal antiserum against JAK2 and She were purchased from Upstate Biotechnology Inc. (Lake Placid, NY), and polyclonal antiserum against TYK2 and STAT3 were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). Polyclonal antiserum against Mpl was generously provided by Zymogenetics (Seattle, WA).

**Cell Lines**—Human sarcoma cell lines were generously provided by George Stark (Cleveland Clinic, OH) and were each maintained in Dulbecco's modified Eagle's medium plus 10% heat-inactivated calf serum, antibiotics (penicillin, streptomycin, fungizone), and l-glutamine. 2C4 (parental) and y2A (JAK2 deficient) lines were grown in the presence of hygromycin, 250 micrograms/ml (Sigma). U1A (TYK2-deficient) cells were selected in neomycin, 1 mg/ml (Sigma). 2C4 and y2A were transfected with a murine Mpl expression vector (pCDNA3-mMpl) by the method of calcium phosphate precipitation. Neomycin-resistant clones were selected by addition of 1 mg/ml G418 and then tested for Mpl expression by Western blot (Fig. 1) and fluorescent activated cell sorter analysis (data not shown). U1A cells were simultaneously transfected with pCDNA3-Mpl and pCMV ouabain, a ouabain resistance cassette. Immunoprecipitations were determined by modified Lowry assay (DC Protein Assay, Bio-Rad); 1 mg of total protein was used for each immunoprecipitation. Lysates were determined by modified Lowry assay (DC Protein Assay, Bio-Rad); 1 mg of total protein was used for each immunoprecipitation. Neomycin-resistant clones were selected in neomycin, 1 mg/ml (Sigma). 2C4 and y2A were transfected with a murine Mpl expression vector (pCDNA3-mMpl) by the method of calcium phosphate precipitation. Neomycin-resistant clones were selected by addition of 1 mg/ml G418 and then tested for Mpl expression by Western blot (Fig. 1) and fluorescent activated cell sorter analysis (data not shown). U1A cells were simultaneously transfected with pCDNA3-Mpl and pCMV ouabain, a ouabain resistance cassette (Pharmingen, San Diego, CA), and clones were selected by addition of 1 μg ouabain (Sigma) to the culture media. Expression of Mpl was confirmed as above.

**Preparation of Lysates**—When 15-cm tissue culture dishes contained a nearly confluent cellular monolayer, the cells were washed twice with phosphate-buffered saline and then incubated 12–16 h in Dulbecco's modified Eagle's medium plus 0.5% bovine serum albumin, antibiotics, and l-glutamine. Matched plates were either unstimulated or exposed for 10 min to 15 ng/ml murine TPO (produced as conditioned media by TPO-secreting baby hamster kidney cells). Cells were scraped off the dishes with rubber spatulas and resuspended at 4°C in 1 ml of lysis buffer. Twenty-five microliters of protein A-agarose beads (Santa Cruz Biotechnology) were combined with 4 μg of poly(di-dc), binding buffer (final concentrations: 15 mM HEPES, pH 7.9, 125 mM NaCl, 8% glycerol; 1 mM diethiothreitol; 0.15 mM EDTA), and the radiolabeled hSIE probe (0.6 pmol). After a 20-min incubation (22°C), the total volume was subjected to electrophoresis on a nondenaturing gel (4% acrylamide; 5% glycerol; 0.5% Triton X-100; 1.5 mM MgCl2, 1 mM EDTA, 100 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 1 mM NaVO4, 1 μg/ml leupeptin, and 1 μg/ml apropin). After 10 min (4°C, occasional vortexing), the particulate material was removed by centrifugation (12,000 × g, 10 min, 4°C), and the cleared lysates were stored at −70°C until use.

**Immunoprecipitation and Western Blot Analysis**—Protein concentrations of lysates were determined by modified Lowry assay (DC Protein Assay, Bio-Rad); 1 mg of total protein was used for each immunoprecipitation. The appropriate antibody was added (2 μg or 2 μl if concentration not available), the volume was adjusted to 1 ml using fresh lysis buffer, and the reactions were gently mixed at 4°C for 2 h. Twenty-five μl of protein A-agarose beads (Santa Cruz Biotechnology) were added for an additional 1-h incubation at 4°C. The beads were then collected by centrifugation, washed three times in cold lysis buffer, and then boiled in sample loading buffer, containing 2% SDS and 1% β-mercaptoethanol. Samples were loaded onto 7.5% acrylamide Laemmli mini-gels with prestained molecular weight markers (Life Technologies, Gaithersburg, MD). Protein was electrophoretically transferred to nitrocellulose which was then blocked in Tris-buffered saline with 0.05% Tween 20 (TBST) and 3% bovine serum albumin. Primary antibodies were used according to manufacturer recommendations at room temperature for 2 h. The membrane was washed three times with TBST (5 min per wash) then incubated with goat anti-mouse or goat anti-rabbit IgG coupled to horseradish peroxidase (1:5000, Bio-Rad, Hercules, CA) for 30 min. After three washes in TBST at 5 min each, chemiluminescent reagents (Santa Cruz Biotechnology) were added for 1 min and the membranes were exposed to x-ray film for 10–90 s. The nitrocellulose was stripped prior to reprobing by incubating at 50°C for 30 min in 62.5 mM Tris (pH 6.8), 100 μM β-mercaptoethanol, and 2% SDS.

**Electrophoretic Mobility Shift Assay**—The human Sis Inducible Element (hSIE; GATCAGATTGCCTAGATGCT), a STAT3-specific double-stranded DNA sequence, was used as a probe for active STAT3 (20). The Prolactin Response Element (PRE; GATCAGATTACTG- GAATTTCAATGCT), was used as a probe for STAT5-specific binding activity (20). The probes were radiolabeled as described previously (18) and were separated from unincorporated [32P]ATP using Centri-Sep columns (Princeton Separations, Princeton, New Jersey). Detergent-free lysates were prepared from unstimulated or TPO-stimulated cells as described previously (18). In a final volume of 10 μl, 1 μg of protein were combined with 4 μg of poly(di-dc), binding buffer (final concentrations: 15 mM HEPES, pH 7.9; 125 mM NaCl; 8% glycerol; 1 mM diethiothreitol; 0.15 mM EDTA), and the radiolabeled hSIE probe (0.6 pmol). After a 20-min incubation (22°C), the total volume was subjected to electrophoresis on a nondenaturing gel (4% acrylamide; 5% glycerol; 0.5% Triton X-100; 1.5 mM MgCl2, 1 mM EDTA, 100 mM NaF, 1 mM phenylmethylsulfonyl fluoride, 1 mM NaVO4, 1 μg/ml leupeptin, and 1 μg/ml aprofin). After 10 min (4°C, occasional vortexing), the particulate material was removed by centrifugation (12,000 × g, 10 min, 4°C), and the cleared lysates were stored at −70°C until use.

**RESULTS**

**JAK2 Is Required for TPO-dependent Tyrosine phosphorylation of Mpl**—Parental cells (2C4/mMpl) or those deficient in either JAK2 (y2A/mMpl) or TYK2 (U1A/mMpl) were either unstimulated or exposed to TPO as described under “Experimental Procedures.” Anti-Mpl antiserum was then used to immunoprecipitate the receptor from whole cellular lysates; at least two independent clones of each cell type were used. Immunoblotting with a phosphotyrosine-specific antibody (4G10) was done to detect TPO-induced Mpl phosphorylation (Fig. 1). We found that the cells with all JAKs present as well as those lacking TYK2 expression were able to phosphorylate Mpl, whereas JAK2-deficient cells were unable to phosphorylate Mpl.

**Phosphorylation of JAK2 Does Not Require TYK2 Expression**—We next wished to examine which of these cell lines sustained TPO-dependent tyrosine phosphorylation of JAK2 and TYK2. First, JAK2 was immunoprecipitated from lysates derived from each cell line either before or after TPO stimulation. These samples were analyzed by Western blot and probed to detect phosphorysotryine incorporation (Fig. 2A). We found that the parental cell lines (2C4-mMpl) demonstrated JAK2 phosphorylation after TPO stimulation, and those cell lines lacking TYK2 expression (y2A-mMpl) had neither phosphorylation nor detectable protein (Fig. 2A, lower strip). However, cell lines deficient in TYK2 expression (U1A-Mpl) retained JAK2 phosphorylation, suggesting that JAK2 activation occurred upstream or independent of TYK2.

![Fig. 1. Mpl phosphorylation in JAK2- and TYK2-deficient cells](image-url)

**FIG. 1.** Mpl phosphorylation in JAK2- and TYK2-deficient cells. Cell lysates were prepared either before (−) or after (+) stimulation with 15 ng/ml TPO for 10 min. Two independently derived clones of cells expressing Mpl were tested for each of the parental (2C4/mMpl), JAK2-deficient (y2A/mMpl), and TYK2-deficient (U1A/mMpl) lines. In each lane, anti-Mpl antiserum was used to immunoprecipitate the receptor from 1 mg of total cell lysate. The resulting Western blot was probed with an anti-phosphotyrosine monoclonal antibody (4G10) to detect tyrosine phosphorylation of Mpl. Below, the blot was stripped and reprobed to demonstrate the presence of Mpl in all lanes. Essentially identical results were obtained from three independent experiments.

JAK2 is required for TPO-dependent tyrosine phosphorylation of Mpl.
probed with a phosphotyrosine-specific antibody (Fig. 2B). Despite a low level of constitutive TYK2 phosphorylation, a marked increase in tyrosine phosphorylation was observed in the TPO-stimulated parental cell lines (2C4-Mpl). As expected, the TYK2-deficient cell lines (U1A-Mpl) had neither detectable TYK2 phosphorylation nor protein. Notably, the JAK2-deficient cell lines (γ2A-Mpl) displayed only low level basal phosphorylation of TYK2, which was not increased by TPO stimulation. This result demonstrates that TPO-induced TYK2 phosphorylation requires JAK2 activity.

**TOPO-dependent Tyrosine Phosphorylation of Shc Requires JAK2**—We next studied which JAK(s) were required for phosphorylation of several downstream signaling proteins. The adapter protein Shc that binds to a phosphotyrosine residue in the carboxyl terminus of Mpl (21, 22) is believed to be directly phosphorylated by JAKs and may play a critical role in TPO-dependent development (21, 23). Immunoprecipitation and Western blot analysis demonstrated that the phosphorylation of all three Shc isoforms occurred in the absence of TYK2 but was not detectable in lysates from JAK2-deficient cells (Fig. 3A). Phosphorylation and Activation of STAT3—In many cell lines and human platelets, both STAT3 and STAT5 are directly tyrosine phosphorylated and activated by Janus kinases (11, 12, 15), but in murine megakaryocytes, STAT5 activation is minimal (18). Using lysates from these cell lines, STAT3 was immunoprecipitated and tested for TPO-dependent tyrosine phosphorylation by Western blot analysis. As for Shc and Mpl, STAT3 phosphorylation was dependent on functional JAK2 protein but not TYK2 (Fig. 3B). To further demonstrate that Mpl was capable of mediating increased STAT3 DNA binding activity, electrophoretic mobility shift assays were performed (Fig. 4). Using the STAT3-specific probe hSIE, TPO-dependent STAT3 binding was seen in the parental and TYK2 nullizygous cells, but not in the JAK2-deficient cells (Fig. 4A). Although STAT5 was readily detected by immunoblotting (data not shown), there was no activation of STAT5 binding activity in any of these cells (Fig. 4B), a pattern of signaling reminiscent of that observed in primary megakaryocytes (18).

**Expression of Functional JAK2 in γ2A/Mpl Cells Restores TPO Signaling**—To prove that JAK2 was essential for TPO-signaling, we expressed wild-type JAK2 in γ2A/Mpl cells (JAK2-deficient) by transfecting them with the expression vector pCDNA3.1 (Zero)-JAK2 (JAK2 cDNA kindly provided by Stuart Frank, Birmingham, AL, and James Ihle, Menlo Park, TN). Cells resistant to Zeocin (500 μg/ml, Invitrogen) were screened for JAK2 expression by Western blot and then tested for TPO-induced phosphorylation of Mpl. When Mpl was immunoprecipitated from γ2A-Mpl and γ2A-Mpl/JAK2 cells, TPO-dependent phosphorylation of the receptor was restored in the cells expressing functional JAK2 (Fig. 5).

### DISCUSSION

It is generally accepted that the activation of Janus kinases in response to ligand binding is essential for signal transduction within the hematopoietic cytokine receptor family. For the interferon-α and interferon-γ receptors, two distinct JAKs are required for signal transduction (4, 5, 12). Studies with the IL-6 receptor indicate that, although three different JAKs are tyrosine phosphorylated after ligand binding, only JAK1 is essential for downstream events (19). Similarly, it was demonstrated that, although epidermal growth factor stimulation activates JAK1, the resulting activation of STAT proteins and induction of c-fos depends only on the intrinsic epidermal growth factor receptor kinase activity (24). Thus, the functional role played by tyrosine kinases cannot be predicted simply by studying the state of phosphorylation or activation. Also, the receptor cytoplasmic domain exhibits specificity regarding which JAKs are recruited to the cell membrane and then activated through receptor subunit aggregation.

One previous study described a mutant form of Mpl that supports TPO-dependent proliferation without phosphorylation of any JAKs, albeit requiring a much higher concentration of TPO (25). However, other reports suggest that mutations leading to the loss of JAK activation result in nonfunctional receptors (14, 22, 26–28). It is possible that additional tyrosine kinase families may be activated by TPO stimulation (29). At present, however, the role of tyrosine kinases other than JAKs has not been defined.

The sarcoma cell lines used in these studies are cytokine-independent and highly primed for autonomous proliferation. Thus, it was not possible to distinguish a proliferative response to TPO in these cell lines (data not shown). Furthermore, as transformed fibroblasts, there was no evident differentiation when grown in TPO for up to 1 week. Specifically, the cells remained adherent with no alteration in morphology or size (data not shown). Nevertheless, the reported data clearly establish that JAK2 is essential for activation of all of the tested secondary molecules implicated in TPO-induced signaling.

In both hematopoietic cell lines and human platelets, JAK2 and TYK2 are tyrosine phosphorylated in response to TPO stimulation. To understand how TPO and Mpl might activate two distinct JAK kinases, we constructed three possible models of the active signaling complex. First, the homodimeric receptor may associate with only one JAK, and the other family member may be phosphorylated as a downstream or incidental event (Fig. 6A). Second, both JAK2 and TYK2 may be able to bind to the box 1/box 2 motifs of Mpl and function interchangeably during TPO signaling (Fig. 6B). Third, there may be another, as yet unidentified, receptor subunit that specifically binds the second kinase molecule (Fig. 6C). From each of these models of the active signaling complex, one can predict the
effect if one of the two kinases were missing. In model B (center), the two kinases would be redundant, and loss of one or the other might cause little change in signal transduction. In the context of a heterodimeric receptor (model C, right), both JAKs would be necessary to form an active signaling complex, and loss of either one would disrupt signaling. Finally, in model A (left), loss of the JAK that directly binds the receptor would disrupt tyrosine phosphorylation, whereas loss of the downstream kinase might have little or no effect.

Our results strongly support the model in Fig. 6A and suggest that JAK2 is essential for TPO-stimulated tyrosine phosphorylation of the receptor (Mpl), both Janus kinases (JAK2 and TYK2), and the downstream effector molecules (STAT3 and Shc). TYK2 is neither sufficient nor necessary for any of these events. Despite the fact that these studies were done in a transformed sarcoma cell system, the early signaling apparatus appears to be intact, and this same system has been used previously to determine the requirements for signal transduction by the interferon, IL-6, and epidermal growth factor receptors (12, 19, 24). Furthermore, our results are strengthened by the fact that signal transduction can be restored in the JAK2-deficient cell line by expression of functional JAK2.
These data reinforce the recent information obtained from the development of JAK2-deficient mice (30). JAK2 knock-out animals were not viable because of failure of embryonic erythrocyte development. However, study of the fetal liver hematopoietic progenitors demonstrated that JAK2 −/− cells were unresponsive to several cytokines, including erythropoietin, thrombopoietin, interleukin-3, and granulocyte-monocyte colony stimulating factor. However, responses to granulocyte colony stimulating factor, interleukin-6, and interferon-α were unaffected because the corresponding receptors utilize other JAK family members. Thrombopoietin and MPL, therefore, belong to the subset of cytokine/receptor pairs that absolutely require JAK2 for signaling activity.

In contrast, there have been no reports of TYK2 knock-out animals, and data from cell lines as well as human platelets suggested that TYK2 is tyrosine phosphorylated in response to TPO. In this report, we establish that TYK2 is a secondary event in thrombopoietin signaling, downstream of JAK2 activation, and not required for the phosphorylation of other signaling molecules. It is possible that TYK2 is phosphorylated either as a substrate of JAK2 or through receptor cross-talk (i.e. MPL activation might activate another associated receptor that itself specifically binds TYK2). However, we have previously demonstrated that TYK2 is not phosphorylated to a significant degree in mature murine megakaryocytes (18). These combined pieces of evidence strongly suggest that the active signaling complex for TPO and MPL contains a homodimeric receptor in which each receptor subunit binds an activated JAK2 molecule.

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REFERENCES
1. Ihle, J. N. (1995) Nature 377, 591–594
2. Ihle, J. N. (1996) Philos. Trans. R. Soc. Lond. B Biol. Sci. 351, 159–166
3. Murakami, M., Narazaki, M., Hibi, M.,Yawata, H., Yasukawa, K., Hamaguchi, M., Taga, T., and Kishimoto, T. (1991) Proc. Natl. Acad. Sci. U. S. A. 88, 11349–11353
4. Leaman, D. W., Leung, S., Li, X., and Stark, G. R. (1996) FASEB J. 10, 1578–1588
5. Briscoe, J., Guschin, D., Rogers, N. C., Watling, D., Muller, M., Horn, F., Heinrich, P., Stark, G. R., and Kerr, I. M. (1996) Philos. Trans. R. Soc. Lond. B Biol. Sci. 351, 167–171
6. Kauhansky, K., Broudy, V. C., Lin, N., Jorgensen, M. J., McCarty, J., Fox, N., Zucker-Franklin, D., and Lofthus-Dox, C. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 3234–3238
7. Broudy, V. C., Lin, N., Fox, N., Taga, T., Saito, M., and Kaushansky, K. (1995) Blood 86, 2026–2032
8. Courtois, G., Benit, L., Mikaeloff, Y., Pauchard, M., Charon, M., Varlet, P., and Gisselbrecht, S. (1995) J. Virol. 69, 2794–2800
9. Alexander, W. S., Metcalf, D., and Dunn, A. R. (1995) EMBO J. 14, 5569–5578
10. Skoda, R. C., Seldin, D. C., Chiang, M. K., Peichel, C. L., Vogt, T. F., and Leder, P. (1993) EMBO J. 12, 2645–2653
11. Jin, L., Sritirarattrakul, N., Emery, D. W., Richard, R. E., Kaushansky, K., Papayannopoulou, T., and Blaz, C. A. (1998) Proc. Natl. Acad. Sci. U. S. A. 95, 8093–8097
12. Darnell, J. E., Jr., Kerr, I. M., and Stark, G. R. (1994) Science 264, 1415–1421
13. Sattler, M., Durstin, M. A., Frank, D. A., Okuda, K., Kaushansky, K., Salgia, R., and Griffen, J. D. (1995) Exp. Hematol. 23, 1040–1048
14. Morita, H., Tahara, T., Matsumoto, A., Kato, T., Miyazaki, H., and Ohashi, H. (1996) FEBS Lett. 395, 228–234
15. Ellumi, Y., Takayama, H., and Okuma, M. (1995) FEBS Lett. 374, 48–52
16. Mu, S. X., Xia, M., Elliott, G., Bogenberger, J., Swift, S., Bennett, L., Lappings, D. L., Hecht, R., Lee, R., and Sarias, C. J. (1995) Blood 86, 4532–4543
17. Tortolani, P. J., Johnston, J. A., Bacon, C. M., McVicar, D. W., Shimozako, A., Linnekin, D., Longo, D. L., and O' Shea, J. J. (1995) Blood 85, 3444–3451
18. Drachman, J. G., Sabath, D. R., Fox, N. E., and Kaushansky, K. (1997) Blood 89, 483–492
19. Guschin, D., Rogers, N., Briscoe, J., Wittnhuhn, B., Watling, D., Horn, F., Pellegrini, S., Yasukawa, K., Heinrich, P., Stark, G. R., Ihle, J. N., and Kerr, I. M. (1995) EMBO J. 14, 1421–1429
20. Twardy, D. J., Wright, T. M., Ziegler, S. F., Baumann, H., Chakraborty, A., White, S. M., Dyer, K. F., and Robin, K. A. (1996) Blood 86, 4409–4416
21. Alexander, W. S., Metcalf, D., and Dunn, A. R. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 557–571
22. Drachman, J. G., and Kaushansky, K. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 2350–2355
23. Hill, R. J., Zeazulya, S., Lu, Y. L., Hollembach, P. W., Joyce Shaikh, B., Bogenberger, J., and Gishizky, M. L. (1996) Cell Growth Differ. 7, 1125–1134
24. Leaman, D. W., Fishardy, S., Flickinger, T. W., Commane, M. A., Schlessinger, J., Kerr, I. M., Levy, D. E., and Stark, G. R. (1996) Mol. Cell. Biol. 16, 369–375
25. Dorsch, M., Fan, P. D., Danial, N. N., Rothman, P. B., and Goff, S. P. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 186, 1947–1955
26. Gurney, A. L., Wong, S. C., Henzel, W. J., and de Sauvage, F. J. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 5292–5296
27. Porteu, F., Rouvez, M. C., Coudert, L., Benit, L., Charon, M., Picard, F., Gisselbrecht, S., Souyri, M., and Dusartier-Fourier, I. (1996) Mol. Cell. Biol. 16, 2473–2482
28. Morella, K. K., Bruno, E., Kumaki, S., Lai, C. F., Fu, J., Wang, H. M., Murray, L., Hoffman, R., Timour, M., Benit, L., Gisselbrecht, S., Zhuang, H., Wojchowski, D. M., Baumann, H., and Gearing, D. P. (1995) Blood 86, 557–571
29. Yamashita, Y., Miyazato, A., Shinmizu, K., Komatsu, N., Miura, Y., Ozawa, K., and Mano, H. (1997) Exp. Hematol. 25, 211–216
30. Pargas, E., Wang, D., Stravopodis, D., Topham, D. J., Marine, J. C., Tegland, S., Yanin, E. P., Bodner, S., Colanomici, O. R., van Deursen, J. M., Grosveld, G., and Ihle, J. N. (1998) Cell 93, 385–395