Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 2285–2289, March 1994
Cell Biology

Activation of JAK2 kinase mediated by the interleukin 6 signal transducer gp130

(tyrosine kinase/cytokine receptor family)

MASASHI NARAZAKI*, BRUCE A. WITTHUHN†, KANJI YOSHIDA*, OLLI SILVENNOINEN†, KIYOSHI YASUKAWA‡, JAMES N. IHLE†, TADAMITSU KISHIMOTO§, AND TETSUYA TAGA*

*Institute for Molecular and Cellular Biology, Osaka University, 1-3 Yamada-oka, Suita, Osaka 565, Japan; †St. Jude Children's Research Hospital, Department of Biochemistry, 332 North Lauderdale, Memphis, TN 38105; ‡Biotechnology Research Laboratory, Tosoh Corporation, 2743-1 Hayakawa, Ayase, Kanagawa 252, Japan; and §Department of Medicine III, Osaka University Medical School, 2-2 Yamada-oka, Suita, Osaka 565, Japan

Contributed by Tadamitsu Kishimoto, December 14, 1993

ABSTRACT **The interleukin 6 receptor-associated signal transducer, gp130, is shared by receptor complexes for leukemia inhibitory factor, oncostatin M, ciliary neurotrophic factor, and interleukin 11. We show here that JAK2 kinase is rapidly tyrosine phosphorylated in mouse embryonic stem cells whose pluripotentiality is maintained only by gp130-sharing cytokines after stimulation that is known to induce gp130 homodimerization. JAK1 is also tyrosine phosphorylated, but to a lesser extent, under the same conditions. Comparable results are obtained with hemopoietic lineage cells such as myeloid leukemic M1 cells and pro-B-cell line-derived transfectants expressing gp130, the former of which differentiate into macrophages after stimulation of gp130 and the latter of which initiate DNA synthesis. gp130-dimerizing stimulus upregulates kinase activity of JAK2 as revealed by immunocomplex kinase assay. Deletion or point mutation in the membrane-proximal cytoplasmic motifs in gp130 that are conserved in the hemopoietic cytokine receptor family results in the loss of tyrosine phosphorylation of JAK2, which coincides with the lack of signal transducing capability of gp130 mutants.**

Receptors and receptor-associated signal transducing components for most of the cytokines functioning in immune and hemopoietic systems are similar in structure and thus belong to the hemopoietic cytokine receptor family (1). Of such receptors and signal transducers in this family, none possesses intrinsic tyrosine kinase domains, unlike conventional growth factor receptors (1–3). However, several lines of evidence have indicated that signaling processes initiated by ligand binding to the receptors in the hemopoietic cytokine receptor family involve tyrosine phosphorylation of receptor components and activation of cellular tyrosine kinases (4, 5). The interleukin 6 receptor (IL-6R)-associated signal transducer, gp130 (6, 7), which is also a member of this family, is shared by other receptor complexes for leukemia inhibitory factor (LIF), oncostatin M, ciliary neurotrophic factor, and IL-11 (2, 5, 8–12). For these cytokines, signaling cascades are believed to be triggered by the formation of homo- or heterodimers of gp130. In the case of the IL-6R system, a complex of IL-6 with IL-6R [or with an extracellular soluble form of IL-6R (sIL-6R)] interacts with gp130 and induces its homodimerization (13). In contrast, heterodimerization of gp130 and LIF-receptor has been shown to be important for signaling of LIF, oncostatin M, and ciliary neurotrophic factor (8, 12). Such homo- or heterodimers including at least one gp130 protein are associated with tyrosine kinase activity, while monomers are not (12, 13). Thus, these dimers look functionally similar to conventional growth factor receptors with intrinsic tyrosine kinases (3) in that ligand-induced dimerization of the cytoplasmic regions of receptor components triggers tyrosine kinase activation.

In the present study, in order to obtain a clue as to a cytoplasmic tyrosine kinase(s) involved in gp130-mediated signal transduction, we have first performed PCR amplification of reverse-transcribed mRNA from mouse embryonic stem (ES) cells by using primers prepared based on the conserved tyrosine kinase motifs. ES cells express gp130 (14, 15) and expression of other cytokine receptors is very limited (16); the pluripotential phenotype of ES cells is known to be maintained only by the gp130-stimulatory factors such as LIF, oncostatin M, and the complex of IL-6 and sIL-6R (15, 17, 18). Based on (*i*) the finding that JAK2 (19, 20) is most frequently observed among potential nonreceptor tyrosine kinases from this first survey and (*ii*) recent reports showing activation of JAK2 upon stimulation with erythropoietin, growth hormone, and IL-3 (20–22), we have examined a role of JAK2 and its close relative, JAK1 (23), in the signaling processes initiated by gp130-homodimerizing reagent—i.e., the IL-6–sIL-6R complex.

MATERIALS AND METHODS

Reverse Transcription and PCR Cloning. Poly(A)$^+$ RNA was prepared from ES cells cultured in the presence of LIF and reverse transcribed by using random hexanucleotide primers (Amersham). The cDNAs were amplified with *Taq* DNA polymerase using degenerative tyrosine kinase-specific primers as described (24), and products of an expected length (≈210 bp) were obtained. The PCR products were cloned in pBluescript plasmid (Stratagene), transfected in *Escherichia coli*, and subjected to DNA sequencing analysis (25).

Antibodies. Anti-JAK1 (no. 1065) and anti-JAK2 (no. 1067) antibodies have been described (20, 21). Anti-JAK2 (no. 683) antibody was prepared in rabbit and guinea pig against a synthetic peptide of murine JAK2 (amino acids: REEDRRTGNPPFIK). Rabbit anti-JAK2 (no. 683) antibody was further affinity purified with the peptide column.

Immunoblot Analysis. Cells were solubilized with Nonidet P-40 (NP-40) lysis buffer (0.5% NP-40/10 mM Tris·HCl, pH 7.6/150 mM NaCl/5 mM EDTA/2 mM Na_3VO_4/1 mM phenylmethylsulfonyl fluoride/5 μg of aprotinin per ml), and clear lysates obtained by centrifugation were incubated with anti-JAK1 or anti-JAK2 antibody. Immunoprecipitates using protein A-Sepharose (Pharmacia) were analyzed by SDS/PAGE and subsequent immunoblotting with anti-phosphotyrosine monoclonal antibody (4G10; Upstate Biotechnology, Lake Placid, NY) using an enhanced chemiluminescence (ECL) detection system (Amersham) according to the manufacturer's procedures.

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: IL-6, interleukin 6; LIF, leukemia inhibitory factor; IL-6R, IL-6 receptor; sIL-6R, soluble IL-6R; ES, embryonic stem.

Immunocomplex Kinase Assay. Cells were solubilized with NP-40 lysis buffer (same as above, except elimination of EDTA). Immunoprecipitates with anti-JAK1 or anti-JAK2 antibody were washed extensively in NP-40 lysis buffer without EDTA and then in kinase buffer (10 mM Hepes, pH 7.4/50 mM NaCl/5 mM $MgCl_2$/5 mM $MnCl_2$/0.1 mM Na_3VO_4). The immunocomplexes were subjected to *in vitro* kinase assay (22) using [γ-^{32}P]-ATP (0.5 mCi/ml; 1 Ci = 37 GBq). Reacted immunocomplexes were washed in NP-40 lysis buffer without EDTA, eluted from Protein A-Sepharose by 50 mM glycine buffer (pH 2.5), and separated by SDS/PAGE. Gels were treated in 1 M KOH at 60°C for 1 hr with gentle agitation (26) and neutralized in 10% acetic acid at 25°C for 2 hr before autoradiography.

RESULTS

We first performed PCR amplification and cloning of tyrosine kinases from reverse-transcribed mRNAs expressed in ES cells whose pluripotentiality is maintained only by gp130-stimulatory cytokines. Among 173 clones encoding tyrosine kinase domains, 28 showed a sequence characteristic to nonreceptor-type tyrosine kinases (27). As shown in Fig. 1*A*, JAK2 was most frequently observed in such tyrosine kinases. We thus examined tyrosine phosphorylation of JAK2 and its close relative JAK1 in ES cells in response to the IL-6–sIL-6R complex, which is known to induce gp130 homodimerization. ES cells were stimulated with IL-6 plus sIL-6R and NP-40 lysates were used for immunoprecipitation and subsequent immunoblot analysis. As shown in Fig. 1*B*, both JAK1 and JAK2 proteins immunoprecipitated by specific antibodies were phosphorylated on tyrosine residues within 10 min after stimulation of gp130 with the IL-6–sIL-6R complex. The extent of tyrosine phosphorylation of JAK2 was more prominent than that of JAK1.

We next examined whether tyrosine phosphorylation of JAK1 and JAK2 after IL-6 stimulation occurs also in hemopoietic lineage cells. Mouse myeloid leukemic M1 cells respond to IL-6 to stop proliferation and differentiate into macrophages (28, 29). Mouse pro-B-cell line (BAF.B03)-derived transfectants expressing human (BAFh130) or mouse (BAFm130) gp130 initiate DNA synthesis in response to the IL-6–sIL-6R complex (7, 14). As shown in Fig. 2*A*, stimulation of either of these cells by IL-6 together with sIL-6R induced tyrosine phosphorylation of JAK1 and JAK2. The phosphorylation of JAK2 was more intense than that of JAK1, as was observed in ES cells. The kinetic analysis showed that tyrosine phosphorylation of JAK1 and JAK2 was very rapid and transient; it was detectable as early as 2 min after stimulation, reached a maximum at 15 min, and then became fainter after 30 min of stimulation (Fig. 2*B*). gp130 protein showed kinetics comparable to its tyrosine phosphorylation after stimulation with IL-6 plus sIL-6R (M.N. and T.T., unpublished observation). Activation of JAK1 and JAK2 kinases upon stimulation of gp130 was further examined. JAK1 and JAK2 proteins were immunoprecipitated by specific antibodies from M1 and BAFh130 cells stimulated

A

Name of kinase	Number of clones
JAK2	12
ETK2	8
YES	3
HCK	2
JAK1	1
FYN	1
LYN	1

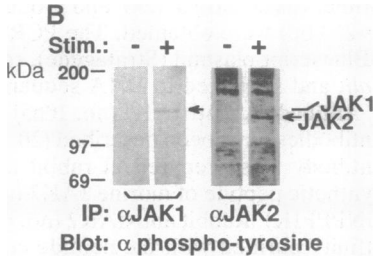

FIG. 1. Identification of potential nonreceptor tyrosine kinases in ES cells and tyrosine phosphorylation of JAK1 and JAK2 after stimulation of gp130. (*A*) Nonreceptor-type tyrosine kinases found in ES cells by reverse transcription PCR cloning and subsequent DNA sequencing. The number of clones having the sequence of each of the indicated kinases is listed. (*B*) Tyrosine phosphorylation of JAK1 and JAK2 in ES cells after gp130 stimulation. ES cells (in twelve 15-cm dishes), which had been cultured with LIF under subconfluent conditions, were starved in serum- and cytokine-free Dulbecco's modified Eagle's medium for 9 hr. Cells were then stimulated with (+) or without (−) a combination of IL-6 (2 μg/ml) plus sIL-6R (2 μg/ml) for 10 min, solubilized with NP-40 lysis buffer, and immunoprecipitated with anti-JAK1 (no. 1065) antiserum or anti-JAK2 (no. 683) rabbit antibody. Immunoprecipitates (IP) were analyzed by SDS/PAGE and subsequent immunoblotting with anti-phosphotyrosine monoclonal antibody.

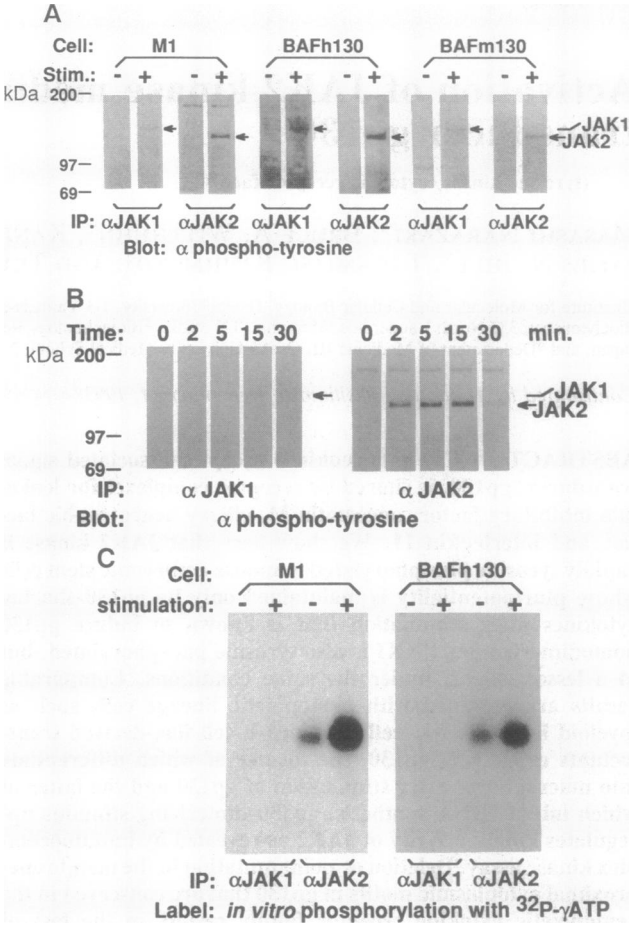

FIG. 2. Tyrosine phosphorylation of JAK1 and JAK2 and activation of JAK2 in myeloid and lymphoid cells after gp130 stimulation. (*A*) gp130-mediated tyrosine phosphorylation of JAK1 and JAK2 in M1 cells and BAF.B03-derived transfectants expressing gp130. Cells (2.4×10^8) were starved in serum-free RPMI 1640 medium for 1 hr (M1) or 3 hr (BAFh130, BAFm130) and stimulated with (+) or without (−) IL-6 plus sIL-6R (same concentrations as in Fig. 1) for 10 min. NP-40 lysates from the cells were subjected to immunoprecipitation with anti-JAK1 (no. 1065) or anti-JAK2 (no. 1067) rabbit anti-serum. SDS/PAGE and immunoblotting were done as described in Fig. 1. (*B*) Kinetic analysis of tyrosine phosphorylation of JAK1 and JAK2. Serum-starved M1 cells were stimulated with IL-6 plus sIL-6R for the indicated time. Tyrosine phosphorylation of JAK1 and JAK2 was analyzed as in *A*. (*C*) Activation of JAK2 after stimulation of gp130. M1 and BAFh130 cells (1.2×10^8 each) were starved, stimulated with (+) or without (−) IL-6 plus sIL-6R, and solubilized in NP-40 lysis buffer lacking EDTA as described. Immunoprecipitates (IP) with anti-JAK1 (no. 211) rabbit anti-serum or anti-JAK2 (no. 683) rabbit antibody were subjected to *in vitro* kinase assay and separated by SDS/PAGE. Gels were alkaline treated and autoradiographed.

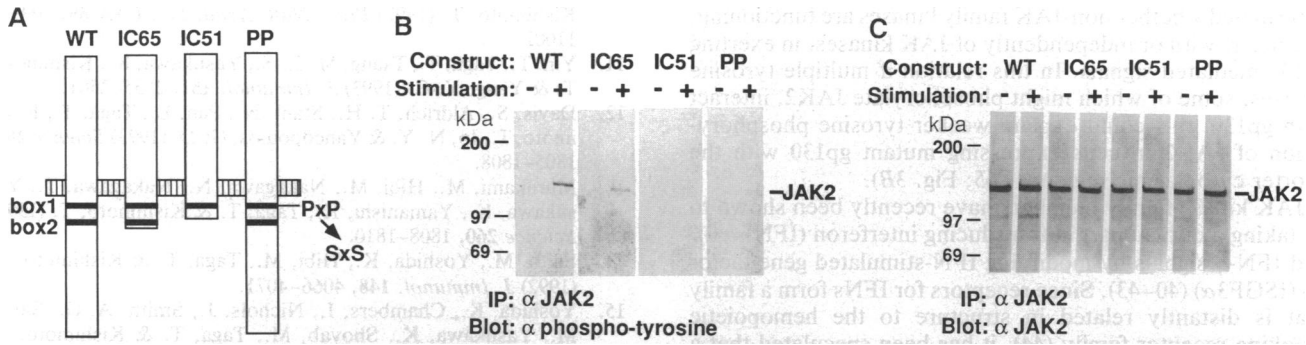

FIG. 3. Involvement of membrane-proximal cytoplasmic motifs of gp130 in inducing tyrosine phosphorylation of JAK2. (*A*) Schematic structure of wild-type and mutant gp130 proteins. (*B*) Tyrosine phosphorylation of JAK2 mediated by gp130 mutants. BAF.B03-derived transfectants (2×10^8 each) expressing wild-type and mutant gp130 proteins were starved, stimulated, and solubilized as in Fig. 2*A*. Immunoprecipitates (IP) with anti-JAK2 (no. 683) rabbit antibody were analyzed by SDS/PAGE and immunoblotting as in Fig. 1. (*C*) Detection of JAK2 protein in BAF.B03 transfectants. Immunoprecipitates prepared as in *B* were analyzed by SDS/PAGE and immunoblotting with anti-JAK2 (no. 683) guinea pig anti-serum.

with IL-6 plus sIL-6R. The immunoprecipitates were subjected to immunocomplex kinase assay, and *in vitro* phosphorylated proteins were separated by SDS/PAGE. As shown in Fig. 2*C*, *in vitro* phosphorylation of JAK2 was significantly upregulated when the protein was prepared from stimulated cells. Phosphorylation of JAK1 *in vitro* could not be detected in this assay.

In the cytoplasmic region of gp130, the membrane-proximal part of ≈60 amino acid residues is structurally similar to that of many other members of the hemopoietic cytokine receptor family. In this part, there are two short and well-conserved stretches of ≈10 amino acids, termed box1 and box2, and in the former, a Pro-Xaa-Pro (PXP) peptide exists in almost all the family members. These motifs have been shown to be critical for signal transduction at least in BAF.B03 cells (30). In BAF.B03-derived transfectants, gp130 mutant protein possessing the membrane-proximal region just enough to contain box1 and box2 (IC65 in Fig. 3*A*, having 65 intracytoplasmic amino acid residues) is sufficient to induce DNA synthesis (ref. 30; M. Murakami, M.N., and T.T., unpublished observation). The gp130 mutant protein having a deletion in box2 (IC51) or having proline-to-serine substitutions in the PXP motif in box1 (PP) cannot mediate such a signal. We examined whether the signal transducing capability of various gp130 mutants correlates with tyrosine phosphorylation of JAK2 kinase after stimulation with IL-6 plus sIL-6R. In BAF.B03-derived transfectants expressing gp130 with the full cytoplasmic region (WT) or with intact box1 and box2 (IC65), the IL-6–sIL-6R complex was shown to induce tyrosine phosphorylation of JAK2, although the IC65-mediated one was weaker than that induced by wild-type gp130 (Fig. 3*B*). Stimulation of IC51 and PP mutants that are incapable of mediating signals in BAF.B03 cells failed to induce tyrosine phosphorylation of JAK2. In all transfectants used in this experiment, a level of immunoprecipitated JAK2 was comparable (Fig. 3*C*). Thus, loss of tyrosine phosphorylation of JAK2 by disruption of conserved box1 and box2 is shown to coincide with the disappearance of biological responses to the IL-6–sIL-6R complex.

DISCUSSION

The complex of IL-6 and IL-6R (or sIL-6R) interacts with gp130 and induces formation of gp130 homodimers, which is associated with tyrosine kinase activity (13). In exerting multiple biological functions of IL-6, homodimerization of the signal transducer gp130 thus appears to be a triggering event. In the present study, we have shown that JAK1 and JAK2 are rapidly and transiently tyrosine phosphorylated in response to IL-6 plus sIL-6R, the gp130-dimerizing stimulus. Activation of JAK2 kinase by this stimulus is also clearly observed by *in vitro* immunocomplex kinase assay. In our experimental system, *in vitro* phosphorylation of JAK1 after a gp130-dimerizing stimulus was undetectable. The possibility remains that activation of JAK1 may take place in intact cells, but it could be below the detection limit when examined by the immunocomplex kinase assay. Another possibility is that JAK2 kinase is activated after gp130 stimulation and JAK1 might be one of its potential substrates. We have also shown that tyrosine phosphorylation of JAK2 was abolished in cells expressing mutant gp130, which could not transduce the signal. The above findings suggest that JAK family kinases may be involved in gp130-mediated signaling. It remains to be determined whether the JAK family members are prerequisite kinases that functionally link the gp130 dimerization to the IL-6-induced biological outputs. To study the possibility of the involvement of the other kinases, we examined the tyrosine phosphorylation of YES, FYN, SRC, and LYN after IL-6 stimulation. In neither U266 myeloma cells nor HepG2 hepatoma cells was tyrosine phosphorylation of these kinases observed. It will be important, however, to examine other nonreceptor kinases including ETK2, which is expressed in ES cells, as well as HCK (see Fig. 1*A*).

Previous studies have demonstrated a physical association of JAK2 with growth hormone receptor and erythropoietin receptor (21, 22). Our studies have not addressed the issue of physical association of gp130 with JAK1 or JAK2, but in initial studies using standard lysis conditions we have not detected JAK1 or JAK2 in immunoprecipitates of gp130, nor have we detected gp130 in immunoprecipitates of JAK1 or JAK2. Since we have shown that membrane-proximal conserved box1 and box2 in gp130 are important for tyrosine phosphorylation of JAK2, it will be necessary to use other approaches to study association and to determine whether the membrane-proximal region is required for gp130–JAK kinase interaction.

IL-6 stimulation was reported to activate RAS and mitogen-activated protein kinases (MAPK) in addition to tyrosine kinases (31–33). NF-IL6, initially identified as a nuclear factor for IL-6 gene expression (34), activates several acute-phase protein genes, which are typical targets of IL-6 (35, 36). Nakajima *et al.* (37) have shown that a RAS-dependent MAPK cascade is essential for NF-IL6 activation. Considering that some of the signals of tyrosine kinase receptors and SRC family kinases reach the nucleus through the RAS-dependent MAPK cascade via adaptor proteins (38, 39), it is important to examine whether JAK family members lead to RAS activation. If not, involvement of non-JAK family kinases in gp130-mediated signaling processes could be speculated as well. Thus, at this moment, it remains to be

determined whether non-JAK family kinases are functioning, in concert with or independently of JAK kinases, in exerting gp130-mediated signals. In this relation if multiple tyrosine kinases, some of which might phosphorylate JAK2, interact with gp130, this could explain weaker tyrosine phosphorylation of JAK2 in cells expressing mutant gp130 with the shorter cytoplasmic region (IC65; Fig. 3*B*).

JAK kinase family members have recently been shown to be taking a critical part in transducing interferon (IFN)-α/-β and IFN-γ signals by modifying IFN-stimulated gene factor 3α (ISGF3α) (40–43). Since receptors for IFNs form a family that is distantly related in structure to the hemopoietic cytokine receptor family (44), it has been speculated that a signaling process initiated by gp130 homodimerization might involve transcription factors related to ISGF3α. Recently, Lütticken *et al.* (45) showed that the acute-phase response factor, a transcription factor that binds to IL-6 response elements in the promoter region of various acute-phase protein genes, is antigenically related to the p91 subunit of ISGF3α, and JAK kinase could activate this factor by the signal through gp130.

IL-6 mediates various biological responses (5). We have shown here that in at least three different types of IL-6-responsive cells, JAK1 and JAK2 kinases are phosphorylated on tyrosine residues very rapidly after stimulation of gp130. Since this stimulation leads to totally different biological outputs (maintenance of pluripotentiality of ES cells, macrophage differentiation of M1 cells, and initiation of DNA synthesis of BAF.B03 transfectants), tyrosine phosphorylation of JAK family kinases may be a general first step in the cytoplasmic signaling cascade just after gp130 dimerization. If so, cell-specific biological responses might be determined by downstream substrates, which may differ from cell type to cell type, for JAK family kinases. Alternatively, phosphorylation and/or activation of a combination of JAK1, JAK2, and possibly another tyrosine kinase might determine cell-specific response. In addition, multiple cytoplasmic signaling pathways (via, for example, RAS/MAPK/NF-IL6 or ISGF3α-related proteins) as discussed above could alternatively determine the specificity. Because the signal transducer gp130 is used in common in receptor complexes as a partner of the receptor component(s) for several other cytokines, it would be of interest to examine what kind of tyrosine kinases and their substrates are functioning in each of these different receptor complexes.

We thank K. Kubota for secretarial assistance. This study was supported by a grant-in-aid for science research from the Ministry of Education, Science, and Culture, Japan, and the Human Frontier Science Program.

1. Bazan, J. F. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 6934–6938.
2. Taga, T. & Kishimoto, T. (1992) *FASEB J.* **6**, 3387–3396.
3. Ullich, A. & Schlessinger, J. (1990) *Cell* **61**, 203–212.
4. Miyajima, A., Kitamura, T., Harada, N., Yokota, T. & Arai, K. (1992) *Annu. Rev. Immunol.* **10**, 295–331.
5. Kishimoto, T., Akira, S. & Taga, T. (1992) *Science* **258**, 593–597.
6. Taga, T., Hibi, M., Hirata, Y., Yamasaki, K., Yasukawa, K., Matsuda, T., Hirano, T. & Kishimoto, T. (1989) *Cell* **58**, 573–581.
7. Hibi, M., Murakami, M., Saito, M., Hirano, T., Taga, T. & Kishimoto, T. (1990) *Cell* **63**, 1149–1157.
8. Gearing, D. P., Comeau, M. R., Friend, D. J., Gimpel, S. D., Thut, C. J., McGourty, J., Brasher, K. K., King, J. A., Gillis, S., Mosley, B., Ziegler, S. F. & Cosman, D. (1992) *Science* **255**, 1434–1437.
9. Ip, N. Y., Nye, S., Boulton, T. G., Davis, S., Taga, T., Li, Y., Birren, S. J., Yasukawa, K., Kishimoto, T., Anderson, D. J., Stahl, N. & Yancopoulos, G. D. (1992) *Cell* **69**, 1121–1132.
10. Taga, T., Narazaki, M., Yasukawa, K., Saito, T., Miki, D., Hamaguchi, M., Davis, S., Shoyab, M., Yancopoulos, G. D. & Kishimoto, T. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 10998–11001.
11. Yin, T., Taga, T., Tsang, M. L.-S., Yasukawa, K., Kishimoto, T. & Yang, Y.-C. (1993) *J. Immunol.* **151**, 2555–2561.
12. Davis, S., Aldrich, T. H., Stahl, N., Pan, L., Taga, T., Kishimoto, T., Ip, N. Y. & Yancopoulos, G. D. (1993) *Science* **260**, 1805–1808.
13. Murakami, M., Hibi, M., Nakagawa, N., Nakagawa, T., Yasukawa, K., Yamanishi, K., Taga, T. & Kishimoto, T. (1993) *Science* **260**, 1808–1810.
14. Saito, M., Yoshida, K., Hibi, M., Taga, T. & Kishimoto, T. (1992) *J. Immunol.* **148**, 4066–4071.
15. Yoshida, K., Chambers, I., Nichols, J., Smith, A. G., Saito, M., Yasukawa, K., Shoyab, M., Taga, T. & Kishimoto, T. (1994) *Mech. Dev.*, in press.
16. Schmitt, R. M., Bruyns, E. & Snodgrass, H. R. (1991) *Genes Dev.* **5**, 728–740.
17. Smith, A. G. (1992) *Semin. Cell Biol.* **3**, 385–399.
18. Smith, A. G. & Hooper, M. L. (1987) *Dev. Biol.* **121**, 1–9.
19. Harpur, A. G., Andres, A.-C., Ziemiecki, A., Aston, R. R. & Wilks, A. F. (1992) *Oncogene* **7**, 1347–1353.
20. Silvennoinen, O., Witthuhn, B. A., Quelle, F. W., Cleveland, J. L., Yi, T. & Ihle, J. N. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 8429–8433.
21. Witthuhn, B. A., Quelle, F. W., Silvennoinen, O., Yi, T., Tang, B., Miura, O. & Ihle, J. N. (1993) *Cell* **74**, 227–236.
22. Argetsinger, L. S., Campbell, G. S., Yang, X., Witthuhn, B. A., Silvennoinen, O., Ihle, J. N. & Carter-Su, C. (1993) *Cell* **74**, 237–244.
23. Wilks, A. F., Harpur, A. G., Kurban, R. R., Ralph, S. J., Zürcher, G. & Ziemiecki, A. (1991) *Mol. Cell. Biol.* **11**, 2057–2065.
24. Wilks, A. F. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 1603–1607.
25. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), 2nd Ed.
26. Cooper, J. A. & Hunter, T. (1981) *Mol. Cell. Biol.* **1**, 165–178.
27. Hanks, S. K., Quinn, A. M. & Hunter, T. (1988) *Science* **241**, 42–52.
28. Miyaura, C., Onozaki, K., Akiyama, Y., Taniyama, T., Hirano, T., Kishimoto, T. & Suda, T. (1988) *FEBS Lett.* **234**, 17–21.
29. Shabo, Y., Lotem, J., Rubinstein, M., Revel, M., Clark, S. C., Wolf, S. F., Kamen, R. & Sachs, L. (1988) *Blood* **72**, 2070–2073.
30. Murakami, M., Narazaki, M., Hibi, M., Yawata, H., Yasukawa, K., Hamaguchi, M., Taga, T. & Kishimoto, T. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 11349–11353.
31. Satoh, T., Nakafuku, M. & Kaziro, Y. (1992) *J. Biol. Chem.* **267**, 24149–24152.
32. Nakafuku, M., Satoh, T. & Kaziro, Y. (1992) *J. Biol. Chem.* **267**, 19448–19454.
33. Daeipour, M., Kumar, G., Amaral, M. C. & Nel, A. E. (1993) *J. Immunol.* **150**, 4743–4753.
34. Akira, S., Isshiki, H., Sugita, T., Tanabe, O., Kinoshita, S., Nishio, Y., Nakajima, T., Hirano, T. & Kishimoto, T. (1990) *EMBO J.* **9**, 1897–1906.
35. Poli, V., Mancini, F. P. & Cortese, R. (1990) *Cell* **63**, 643–653.
36. Isshiki, H., Akira, S., Sugita, T., Nishio, Y., Hashimoto, S., Pawlowski, T., Suemastu, S. & Kishimoto, T. (1991) *New Biol.* **3**, 63–70.
37. Nakajima, T., Kinoshita, S., Sasagawa, T., Sasaki, K., Naruto, M., Kishimoto, T. & Akira, S. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 2207–2211.
38. Egan, S. E., Giddings, B. W., Brooks, M. W., Buday, L., Sizeland, A. M. & Weinberg, R. A. (1993) *Nature (London)* **363**, 45–51.
39. Li, N., Batzer, A., Daly, R., Yajnik, V., Skolnik, E., Chardin, P., Bar-Sagi, D., Margolis, B. & Schlessinger, J. (1993) *Nature (London)* **363**, 85–88.
40. Velazquez, L., Fellous, M., Stark, G. R. & Pellegrini, S. (1992) *Cell* **70**, 313–322.
41. Fu, X.-Y. (1992) *Cell* **70**, 323–335.
42. Müller, M., Briscoe, J., Laxton, C., Guschin, D., Ziemiecki, A., 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.
43. Watling, D., Guschin, D., Müller, M., Silvennoinen, O., Witthuhn, B. A., Quelle, F. W., Rogers, N. C., Schindler, C., Stark, G. R., Ihle, J. N. & Kerr, I. M. (1993) *Nature (London)* **366,** 166–170.
44. Bazan, J. F. (1990) *Cell* **61,** 753–754.
45. Lütticken, C., Wegenka, U. M., Yuan, J., Buschmann, J., Schindler, C., Ziemiecki, A., Harpur, A. G., Wilks, A. F., Yasukawa, K., Taga, T., Kishimoto, T., Barbieri, G., Pellegrini, S., Sendtner, M., Heinrich, P. C. & Horn, F. (1994) *Science* **263,** 89–92.