Direct Association of STAT3 with the IFNAR-1 Chain of the Human Type I Interferon Receptor

(Received for publication, October 19, 1995, and in revised form, February 9, 1996)

Chuan-He Yang‡, Wei Shi‡, Leela Basut, Aruna Murti, Stefan N. Constantinescu‡, Lawrence Blatt‡, Ed Crozet, Jerald E. Mullersman‡, and Lawrence M. Pfeffer‡

From the Department of Pathology, University of Tennessee Health Science Center, Memphis, Tennessee 38163, and Department of Protein Biochemistry and Biophysics, Berlex Biosciences, Inc., Richmond, California 94804

Cytokines are multifunctional mediators of the growth and differentiation of hematopoietic, lymphopoietic, and neural systems. They exert their effects through specific surface receptors expressed on target cells, triggering biological effects through the activation of specific gene transcription. One approach to the identification of cis-responsive elements withingenesandthetranscriptionfactorsresponsiveactivation by cytokines is the identification of cis-responsive activation motifs. For example, the type I interferons (IFNs), 1 elementswithingenescalaregenesandthetranscriptionfactorsresponsiveactivation by cytokines is the identification of cis-responsive activation motifs. For example, the type I interferons (IFNs), 1 elementswithingenescalaregenesandthetranscriptionfactorsresponsiveactivation by cytokines is the identification of cis-responsive activation motifs. For example, the type I interferons (IFNs), 1

*This work was supported by National Institutes of Health Grant GM36716 and by a grant from AMGEN, Inc., Thousand Oaks, California 91320, and Department of Protein Biochemistry and Biophysics, Berlex Biosciences, Inc., Richmond, California 94804.

MATERIALS AND METHODS

Cdls—Human Daudi and U266 cells were maintained in 2.5–10 5 cells/ml in RPMI 1640 containing 10% defined bovine calf serum (HyClone). Human HeLa-a cells were grown in stirred suspension culture in spinner medium supplemented with 10% bovine calf serum.

IFN and mAbs—The activity of recombinant human IFNα (IFN- Con1), provided by AMGEN, is expressed in international reference units (IU)/ml as assayed by protection against the cytopathic effect of vesicular stomatitis virus on human fibroblasts, using the NIH human IFNα standard for reference. mAbs directed against the extracellular domain of the IFNAR-1 chain have been described previously (6).

Nuclear Extracts and Gel Shift Assays—Nuclei were extracted with buffer containing 20 mM Tris-HCl, pH 7.85, 250 mM sucrose, 0.4 mM KCl, 1.1 mM MgCl2, 5 mM β-mercaptoethanol, and 0.4 mM phenylmethylsulfonyl fluoride, and extracts were frozen on dry ice and stored at −80°C

Direct Association of STAT3 with the IFNAR-1 Chain of the Human Type I Interferon Receptor

Based on the reports of the activation of the transcription factor known as STAT3 (for signal transducers and activators of transcription) or APRF (for acute phase response factor) by various cytokines, we investigated the possible role of STAT3 in type I interferon (IFN) receptor signaling. We show that STAT3 undergoes IFNα-dependent tyrosine phosphorylation and IFNα treatment induces protein-DNA complexes that contain STAT3. In addition, STAT3 associates with the IFNAR-1 chain of the type I receptor in a tyrosine phosphorylation-dependent manner upon IFNα addition. The binding of STAT3 to the IFNAR-1 chain occurs through a direct interaction between the SH2 domain-containing portion of STAT3 and the tyrosine-phosphorylated IFNAR-1 chain. Furthermore, tyrosine-phosphorylated STAT3 bound to the IFNAR-1 chain also undergoes a secondary modification involving serine phosphorylation. This phosphorylation event is apparently mediated by protein kinase C, since it was blocked by low concentrations of the protein kinase inhibitor H-7. The biological relevance of IFN activation of STAT3 is further illustrated by the finding that STAT3 is not activated by IFN in a cell line resistant to the antiviral and anti-proliferative actions of IFNα but in which other components of the JAK-STAT pathway are activated by IFNα.

and modulation of the immune system. IFN signaling to the cell nucleus involves the tyrosine phosphorylation of STAT (signal transducers and activators of transcription) proteins. IFN-activated STAT1 and STAT2 translocate to the nucleus, where they recognize the conserved IFN stimulus response element (ISRE) within the promoter of ISGs, which is both necessary and sufficient for ISG transcription (2, 3). Central to the type I IFN-activated pathway are two non-receptor protein tyrosine kinases, JAK1 and TYK2, which apparently mediate the tyrosine phosphorylation of IFN receptor chains and STATs (4, 5).

The intracellular domains of type I IFN receptor chains contain conserved motifs that likely function in transmembrane signaling (6, 7). The ligand-induced tyrosine phosphorylation of STAT transcription factors is one of the events most proximal to cytokine-dependent JAK activation (2, 3). Recent studies indicate that many cytokines, including type I IFNs, induce tyrosine phosphorylation of the STAT3 transcription factor (8, 9), also known as the acute phase response factor (APRF) involved in acute phase gene expression. We previously proposed that the intracellular domain of the IFNAR-1 chain plays a critical role in type I IFN signaling by specifically docking important SH2 domain-containing cytoplasmic proteins (6). Therefore, we investigated the possible role of STAT3 in IFNα signaling through the IFNAR-1 chain.

The type I IFN receptor apparently consists of multiple glycoprotein subunits (6, 10, 11). The cDNAs coding for two subunits, the IFNAR-1 and IFNAR-2 chains, have recently been cloned (12–15). We show that STAT3 associates with the IFNAR-1 chain in a tyrosine phosphorylation-dependent manner after exposure of cells to IFN. The binding of STAT3 to the IFNAR-1 chain can occur through a direct interaction between the SH2 domain-containing portion of STAT3 and tyrosine-phosphorylated IFNAR-1 chain. The biological significance of IFN activation of STAT3 is further borne out by the finding that STAT3 is the only signaling molecule in the JAK-STAT pathway not activated by IFNα in an IFNα-resistant cell line.

Cytokines are multifunctional mediators of the growth and differentiation of hematopoietic, lymphopoietic, and neural systems. They exert their effects through specific surface receptors expressed on target cells, triggering biological effects through the activation of specific gene transcription. One approach to the understanding of the molecular basis of transcriptional activation by cytokines is the identification of cis-responsive elements within genes and the transcription factors responsive to cytokine signals. For example, the type I interferons (IFNs), IFNα and IFNβ, induce the transcription of the early IFN-stimulated gene (ISG) gene family (1). IFNs are cytokines that have profound effects on cells, including antiviral protection, inhibition of the proliferation of normal and transformed cells, and modulation of the immune system. IFN signaling to the cell nucleus involves the tyrosine phosphorylation of STAT (signal transducers and activators of transcription) proteins. IFN-activated STAT1 and STAT2 translocate to the nucleus, where they recognize the conserved IFN stimulus response element (ISRE) within the promoter of ISGs, which is both necessary and sufficient for ISG transcription (2, 3). Central to the type I IFN-activated pathway are two non-receptor protein tyrosine kinases, JAK1 and TYK2, which apparently mediate the tyrosine phosphorylation of IFN receptor chains and STATs (4, 5).
Fig. 1. The presence of STAT-related proteins in ISRE and SIE protein-DNA complexes induced by IFN. A, nuclear extracts were prepared from control and IFN-treated (5,000 IU/ml) IFN-sensitive Daudi cells and then subjected to EMSA with a 32P-labeled ISRE or SIE probe in the absence or presence of a 50-fold excess of unlabeled oligonucleotide probes. In addition, one set of nuclear extracts from IFN-treated cells was preincubated with αSTAT3 prior to EMSA analysis. The positions of ISGF complexes and the SIE complexes are indicated. B, nuclear extracts from IFN-treated (5,000 IU/ml) Daudi cells were incubated with normal rabbit serum (NRS), αSTAT1, αSTAT2, or αSTAT3 prior to EMSA analysis with a 32P-labeled SIE probe.

(16–18). For gel shift analysis, the nuclear extracts were incubated with a 32P-end-labeled promoter probe for either the high affinity sis-inducible element (SIE) from the c-fos gene (5’-AGCTCTTCTTGCTATG-5’) or the ISRE from ISG15 (5’-GATCCATGCCTCGG-GAAGGGAAACCGAAACTGAAGCC-3’) (19, 20) at 25 °C for 30 min, and the free probe was separated from protein-DNA complexes on 5% polyacrylamide gels. For supershift assays, nuclear extracts were preincubated with a 1:50 dilution of normal rabbit serum, anti-STAT1 (αSTAT1, Santa Cruz Laboratories), anti-STAT2 (αSTAT2, Santa Cruz Laboratories), or anti-STAT3 (αSTAT3) at 25 °C for 0.5 h. Gels were quantitated by PhosphorImager autoradiography.

GST Fusion Construct—A segment encoding residues 498–770 from a human STAT3 cDNA was subcloned into the EcoRI and SacI sites of pGEX-KG (21). The construct was confirmed by restriction enzyme digestion. The fusion protein (STAT3-GST) was obtained from Escherichia coli transformed with the plasmid construct and affinity-purified on glutathione-Sepharose (Pharmacia Biotech Inc.) as described previously (22).

Immunoprecipitations and Immunoblot Analysis—For immunoprecipitation studies, 1 × 10⁷ cells were treated with IFNα (5,000 IU/ml) at 37 °C for the indicated periods of time and then washed with ice-cold phosphate-buffered saline and lysed for 20 min in lysis buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.5% Nonidet P-40, 15% glycerol) containing 1 mM NaF, 1 mM Na3VO4, 1 mM phenylmethylsulfonyl fluoride, 5 μg/ml soybean trypsin inhibitor, 5 μg/ml leupeptin, and 1.75 μg/ml benzamidine (3). Samples were centrifuged (12,000 × g, 15 min) at 4 °C, and supernatants were immunoprecipitated with αSTAT3, anti-phosphotyrosine (α Tyr(P), Oncogene Sciences Ab-2), or anti-IFNAR-1 (αIFNAR-1) overnight at 4 °C. Immune complexes were collected using Protein A-Sepharose beads (Pharmacia) and eluted in sample buffer. Samples were run on SDS, 7.5% PAGE, transferred to PVDF membranes (Millipore), and probed with αSTAT3 mAb (dilution, 1:1000), followed by anti-mouse IgG coupled with horseradish peroxidase (Amersham Corp.). Blots were developed using enhanced chemiluminescence (ECL, Amersham Corp.).

For precipitation with GST fusion proteins, lysates from control or IFN-treated Daudi cells were precipitated with STAT3-GST or GST bound to glutathione-agarose beads. The precipitated proteins were resolved by SDS-PAGE (7.5%), blotted onto PVDF membranes, and probed with αIFNAR-1. For blotting with GST fusion proteins, αIFNAR-1 immunoprecipitates resolved by SDS-PAGE were probed with STAT3-GST or GST (purified after elution with glutathione from agarose beads). The IFNAR-1 chain was visualized by ECL using a hamster αGST mAb and a goat anti-hamster IgG horseradish peroxidase conjugate (Southern Biotechnology Associates).

RESULTS

IFNα Induction of STAT-related Proteins That Bind to Specific Promoter Elements—Nuclear extracts prepared from IFN-sensitive Daudi cells treated with IFNα were incubated with a labeled probe for either the high affinity SIE or the ISRE, and the resultant DNA-protein complexes were analyzed by an electrophoretic mobility shift assay (EMSA). Fig. 1 shows that treatment with IFNα induced the STAT-related DNA binding factors, sis-inducible factor (SIF)-A, SIF-B, and SIF-C, as well as IFN-stimulated gene factors (ISGFs). No DNA binding to the SIE was detected in the presence of excess unlabeled SIE oligonucleotide, and binding was not competed by an excess of ISRE oligonucleotide. Similarly, no DNA binding to the ISRE probe was detected in the presence of excess ISRE oligonucleotide, and binding was not competed by the SIE oligonucleotide. Taken together these results indicate that the binding to each probe was specific. To detect specific STAT proteins in the IFN-inducible DNA-protein complexes, we performed gel supershift assays with various STAT-specific antisera (αSTAT). Previous studies showed that the ISRE-specific ISGF3 factor contains only STAT1 and STAT2 but not STAT3 (1). Consistent with these results, we found that the ISGF3 complex was not supershifted with αSTAT3 but was supershifted by either αSTAT1 or αSTAT2 (data not shown). However, a distinct slowly migrating DNA-protein complex formed with the ISRE, denoted ISGFX in Fig. 1, was supershifted by αSTAT3. In addition, αSTAT3 supershifted SIF-A and SIF-B formed with the SIE in IFN-treated Daudi cells, while αSTAT1 supershifted SIF-C (23, 24). Neither control normal rabbit serum nor αSTAT2 shifted any of the IFN-induced SIE DNA-protein complexes. Thus, IFNα treatment of Daudi cells induced DNA binding activities attributable to STAT1, STAT2, and STAT3, and possibly additional unknown factor(s).

IFNα-dependent Tyrosine Phosphorylation of STAT3 and the Coprecipitation of STAT3 with the IFNAR-1 Chain—Tyrosine phosphorylation of STATs is essential for their DNA binding activity and their translocation from the cytoplasm into the nucleus (1). To determine whether STAT3 is tyrosine-phosphorylated in response to IFN, cell lysates were precipitated with αSTAT3 and analyzed by blotting with αTyr(P) (Fig. 2). Although similar amounts of STAT3 from both IFN-treated and untreated cells were precipitated with αSTAT3, only αSTAT3 precipitates from IFN-treated cells contained tyrosine-phosphorylated STAT3. Furthermore, a doublet of STAT3 was observed, as illustrated in Fig. 2B. The more slowly migrating STAT3 band reportedly represents a secondary modification of STAT3 involving serine phosphorylation (25, 26).

We have previously shown that the IFNAR-1 chain underlies IFN-dependent tyrosine phosphorylation (6). In addition, several tyrosine-phosphorylated proteins coprecipitate with the IFNAR-1 chain. To determine if STAT3 coprecipitated with IFNAR-1, lysates from control and IFN-treated Daudi cells were precipitated with αIFNAR-1 and analyzed by blotting.
with αSTAT3 (Fig. 2). Although similar amounts of IFNAR-1 chain were precipitated by αIFNAR-1 (data not shown), only αIFNAR-1 precipitates from IFN-treated cells contained STAT3 protein (Fig. 2). Furthermore, blotting with αTyr(P) indicated that the kinetics of tyrosine phosphorylation of IFNAR-1 and STAT3 were remarkably similar. IFN-dependent coprecipitation of STAT3 with the IFNAR-1 chain was also observed in HeLa epithelioid carcinoma cells and U-266 lymphoblastoid cells (data not shown). As observed in αSTAT3 precipitates, the STAT3 band precipitated by αIFNAR-1 resolved as a doublet. This result directly places the kinase responsible for the secondary modification of STAT3 (serine phosphorylation) in close proximity to the IFNAR-1 chain of the receptor.

The Effects of PKC Inhibitors on IFN-induced Serine Phosphorylation of STAT3—Recently, it has been shown that IFN also induces the serine phosphorylation of both STAT1 and STAT3 (25, 26), an event that is necessary for maximal activation of transcription (26). Since we previously showed that IFNα activates the ε and δ subspecies of PKC in IFN-sensitive Daudi cells and that PKC plays a role in IFNα action in these cells (27), we investigated the role of PKC in the serine phosphorylation of STAT3. The serine phosphorylation of STAT3 is readily detectable because it results in the presence of a doublet of tyrosine-phosphorylated STAT3 in SDS-PAGE (Fig. 2). Since at low concentrations H-7 is a selective inhibitor of PKC (29), we examined the effect of pretreatment of Daudi cells with various concentrations of H-7 on the IFN-induced tyrosine-phosphorylated STAT3 doublet. As shown in Fig. 2B, H-7 caused a dramatic disappearance of the slower mobility, upper STAT3 band at concentrations of 10 μM or less, with a 50% inhibition observed at 3 μM, which is consistent with an inhibitory effect of H-7 on PKC. In addition, pretreatment with the PKC inhibitor staurosporine also blocked the secondary modification of STAT3, and thus only the faster mobility tyrosine-phosphorylated form of STAT3 is detected. At high concentrations staurosporine (200 nM) and H-7 (30 μM) also block the tyrosine phosphorylation of JAK protein tyrosine kinases and thus inhibit the tyrosine phosphorylation of STAT3 (Fig. 2B). Thus, these results suggest that an IFN-activated PKC may mediate the serine phosphorylation of STAT3. We are currently evaluating whether PKC subspecies or other serine kinases are physically associated with the type I IFN receptor.

Precipitation and Direct Blotting of the Tyrosine-phosphorylated IFNAR-1 Chain by a STAT3-GST Fusion Protein—STAT3 contains an SH2 domain (3), a modular noncatalytic domain of about 100 amino acid residues, that mediates high affinity interactions of cytoplasmic effectors with specific phosphotyrosine motifs in activated cell surface receptors (30, 31). The phosphorylation of conserved motifs present in cytokine receptor subunits creates multifunctional docking sites for SH2 domain-containing cytoplasmic effectors (6, 32, 33). Since the IFNAR-1 chain undergoes rapid tyrosine phosphorylation and associates with STAT3, one likely possibility is that this interaction involves the SH2 domain of STAT3. Although the phosphopeptide specificity of many SH2 domain-containing proteins has been determined, attempts to define the phosphopeptide specificity of the SH2 domains of STATs have been unsuccessful (34). Recent structural and functional studies on the Src family tyrosine kinases Lck and Fyn have demonstrated important interactions between SH2 domains and adjacent protein structures (i.e. SH3 domains) (35, 36). Therefore, we prepared a GST fusion protein (STAT3-GST) that encompasses the SH2 and SH3 domains of STAT3, as well as carboxyl-terminal residues. Lysates were prepared from IFN-treated Daudi cells and incubated with STAT3-GST bound to glutathione-agarose beads. The material bound was analyzed by blotting with αIFNAR-1. As shown in Fig. 3, IFN treatment resulted in binding of the IFNAR-1 chain to the SH2 domain-containing STAT3-GST. Pretreatment of cells with genistein, α

2 L. M. Peffer, M. K. Dahmer, C. Wang, R. Pine, N. C. Reich, A. Murti, D. J. MacEwan, and S. N. Constantinescu, submitted for publication.

3 C.-H. Yang and L. M. Peffer, unpublished observations.
tyrosine kinase inhibitor, blocked interaction of the IFNAR-1 chain with STAT3 in IFN-resistant Daudi cells, demonstrating that tyrosine phosphorylation of IFNAR-1 is required for interaction with the fusion protein. The specificity of the interaction is further illustrated by the finding that IFNAR-1 chain was not bound to GST protein alone. To show that STAT3 can directly interact with the tyrosine-phosphorylated IFNAR-1 chain, IFNAR-1 precipitates were blotted with STAT3-GST. STAT3-GST bound only to tyrosine-phosphorylated IFNAR-1 chain, since binding was only detected after IFN treatment and was abolished by pretreatment with genistein. In contrast, blotting with GST fusion proteins containing the SH2 domains of Abl, Crk, or GTPase-activating protein did not show any interaction with tyrosine-phosphorylated IFNAR-1 chains (data not shown).

**Fig. 4.** The failure of IFN<sub>α</sub> to activate STAT3 in IFN-resistant Daudi cells. A, lysates prepared from control or IFN-treated (5,000 IU/ml) cells were precipitated with αSTAT3. The proteins were resolved by SDS-PAGE, blotted onto PVDF membranes, and probed with αTyr(P) or αSTAT3. B, nuclear extracts from Daudi cells treated with IFN<sub>α</sub> (5,000 IU/ml) were preincubated with αSTAT1, αSTAT2, or αSTAT3 and then subjected to EMSA with a 32P-labeled SE1 probe. The positions of SIF complexes formed in extracts from IFN-sensitive cells are presented for reference. gen, genistein.

**DISCUSSION**

The binding of cytokines to cell surface receptors on target cells induces the transcription of specific sets of genes. This new gene expression involves the cytokine-induced tyrosine phosphorylation of specific subsets of STAT transcription factors and the formation of phosphorylation-dependent STAT complexes. The mechanism by which a cytokine and its cognate receptor selectively activate only certain STATs is poorly understood, but several lines of evidence indicate that it involves intermediate phosphotyrosine-, SH2 domain-dependent complexes between the receptor chain and specific STAT factors. For example, IFN<sub>γ</sub> induces the tyrosine phosphorylation of the IFNGR-1 chain of the multisubunit IFN<sub>γ</sub> receptor as well as of STAT1 (37). A functionally critical tyrosine residue in the membrane distal region of the IFNGR-1 chain is involved in STAT1 activation. Furthermore, phosphopeptides corresponding to this region interact with STAT1 and block its activation. These results were the first to show that a specific tyrosine-based activation motif (TBM) in the cytosolic tail of an IFN receptor subunit dictated specific STAT activation. The results reported herein establish that IFN<sub>α</sub> activates STAT3 directly through an interaction between the tyrosine-phosphorylated IFNAR-1 chain and the SH2 domain-containing half of STAT3. It is likely that STAT3 becomes tyrosine-phosphorylated by the receptor-associated Jak1 or Tyk2 kinases. Most importantly, we demonstrate a direct association of a STAT with a cytokine receptor chain and thus provide a mechanism whereby cytokine receptors dictate signaling specificity.

The IFN<sub>α</sub> chain of the type I IFN receptor undergoes ligand-dependent tyrosine phosphorylation and plays a crucial role in signal transduction (6, 38, 39). The cytoplasmic tails of the mouse, human, and bovine IFNAR-1 chains contain a perfectly conserved membrane distal amino acid motif, KYSQTSQDNSGYSE (6, 7). We previously proposed that this TBM plays a critical role in the signaling of type I IFN through its receptor by specifically docking important SH2 domain-containing cytoplasmic proteins (6). Recently, it was reported that a TBM of YXXQ in the cytosolic tail of the shared signal-transducing gp130 chain of the IL6 receptor family is required for cytokine-dependent STAT3 activation (40). Thus, it is possible that the conserved YXXQ motif of the cytosolic tail of the IFNAR-1 chain may also serve as a docking site for STAT3. We are presently investigating whether this motif in fact is the STAT3 docking site on the IFNAR-1 chain.

STAT3 also undergoes serine phosphorylation (25, 26), a modification that was blocked in Daudi cells by serine kinase inhibitors (H-7 and staurosporine) in IFN-treated cells. Thus, we can place both serine and tyrosine protein kinases in the vicinity of the type I IFN receptor and in early transmembrane signaling events. Furthermore, the IC<sub>50</sub> for inhibitory action of H-7 on IFN-induced STAT3 serine phosphorylation suggests that PKC may mediate the serine phosphorylation of STAT3. We have previously shown that, although Daudi cells express PKCa, -β, -δ, -ε, -ζ, and -θ, IFN<sub>α</sub> selectively activates PKCδ and PKCe (27). Thus, it is tempting to suggest that either one or both IFN-activated PKC subspecies may mediate the phosphorylation of STAT3. Recently, it has been shown that the serine phosphorylation of STAT1 and STAT3 is necessary for maximal activation of transcription (26).

Finally, although much attention has been focused on the role of STAT1 and STAT2 in IFN<sub>α</sub> action, our results suggest that STAT3 is also involved in the biological actions of IFN. We previously reported that IFN<sub>α</sub> rapidly induces ISG transcription in IFN-sensitive and IFN-resistant Daudi cells (17, 28). However, while ISG transcription persists at high levels in the IFN-sensitive Daudi line, the activation of ISG transcription is only transient in IFN-resistant Daudi cells. IFN-resistant Daudi cells undergo the normal activation of other components of the Jak-STAT pathway, as determined by the ligand-dependent tyrosine phosphorylation of STAT1, STAT2, Jak1, and Tyk2. It is of note that the ligand-dependent tyrosine phosphorylation of the IFNAR-1 chain was reduced in IFN-resistant Daudi cells when compared with IFN-sensitive cells. In the present report we show that, although IFN activates STAT3 in IFN-sensitive Daudi cells, STAT3 was not activated in the IFN-resistant line when assayed by STAT3 tyrosine phosphorylation or by formation of STAT3-containing protein-
DNA complexes in gel supershift assays. We provide evidence for a direct phosphotyrosine-dependent interaction between the IFNAR-1 chain of the human type I interferon receptor and the SH2 domain-containing portion of STAT3. This association is required for the formation of functional STAT3-containing transcription factors in response to interferon-γ.

Acknowledgments—We thank Drs. J. E. Darnell, Jr. (Rockefeller University) for providing αSTAT3 and B. Mayer (Harvard University) for providing GST fusion proteins containing the SH2 domains of Abl, Crk, or GTPase-activating protein.

REFERENCES
1. Darnell, J. E., Jr., Kerr, I. M., and Stark, G. R. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 6376–6380
2. Schindler, C., Shuai, K., Prezioso, V. R., and Darnell, J. E. (1992) Science 257, 809–813
3. Fu, X.-Y. (1992) Cell 70, 323–335
4. Muller, M., Briscoe, J., Laxton, C., Guschin, D., Ziemiecki, A., Silvennoinen, O., Harpur, A. G., Barbieri, G., Witthuhn, B. A., Schindler, C., Pelligrini, S., Wilks, A. F., Ihle, J. N., Stark, G. R., and Kerr, I. M. (1993) Nature 366, 129–135
5. Velazquez, L., Fellous, M., Stark, G. R., and Pelligrini, S. (1992) Cell 70, 313–322
6. Constantinescu, S. N., Croze, E., Wang, C., Murti, A., Basu, L., Mullersman, J. E., and Pfeffer, L. M. (1994) Proc. Natl. Acad. Sci. U.S.A. 91, 9602–9606
7. Mullersman, J. E., and Pfeffer, L. M. (1994) Trends Biochem. Sci. 20, 55–56
8. Akira, S., Nishio, Y., Inoue, M., Wang, X.-J., Wei, S., Matsusaka, T., Yoshida, K., Sudo, T., Naruto, M., and Kishimoto, T. (1994) J. Immunol. 153, 2645–2651
9. Zhong, Z., Wen, Z., and Darnell, J. E., Jr. (1994) Science 264, 4806–4810
10. Vanden Broecke, C., and Pfeffer, L. M. (1988) J. Interferon Res. 8, 303–311
11. Platanias, L. C., Pfeffer, L. M., Cruciani, R., and Colamonici, O. R. (1993) J. Immunol. 150, 3382–3388
12. Ueno, G., Luttigall, G., and Gresser, I. (1990) Cell 60, 225–234
13. Novick, D., Cohen, B., and Rubinstein, M. (1994) Cell 77, 391–400
14. Domanski, P., Witte, M., Kellum, M., Rubinstein, M., Hackett, R., Pitha, P., and Colamonic, O. R. (1995) J. Biol. Chem. 270, 21606–21611
15. Luttigall, G., Holland, S. J., Cinato, E., Monneron, D., Rebuli, J., Rogers, N. C., Smith, J. M., Stark, G. R., Gardiner, K., Mogensen, K. E., Kerr, I. M., and Uzel, G. (1995) EMBO J. 14, 5100–5108
16. Improta, T., Pine, R., and Pfeffer, L. M. (1992) J. Interferon Res. 12, 87–94
17. Kester, D. S., Pine, R., Pfeffer, L. M., Levy, D. E., and Darnell, J. E. (1988) EMBO J. 7, 3779–3783
18. Levy, D. E., Kessler, D. S., Pine, R., and Darnell, J. E. (1989) Genes & Dev. 3, 1362–1371
19. Reich, N. C., Evans, B., Levy, D. E., Fahey, D., Knight, E., and Darnell, J. E. (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 6394–6398
20. Wagner, B. J., Hayes, T. E., Coban, C. J., and Cohran, B. H. (1990) EMBO J. 9, 4477–4484
21. Guan, K., and Dixon, J. E. (1991) Anal. Biochem. 192, 262–267
22. Smith, D. B., and Johnson, K. S. (1988) Gene (Amst.) 67, 31–40
23. Sadowski, H. B., Shuai, K., Darnell, J. E., Jr., and Gilman, M. Z. (1993) Science 261, 1739–1743
24. Zhong, Z., Wen, Z., and Darnell, J. E., Jr. (1994) Science 264, 95–98
25. Boulton, T., Zhong, Z., Wen, Z., Darnell, J. E., Jr., Stahl, N., and Yanopoulos, G. D. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 6915–6919
26. Wen, Z., Zhong, Z., and Darnell, J. E., Jr. (1995) Cell 82, 241–250
27. Pfeffer, L. M., Eisenkraft, B. L., Reich, N. C., Improta, T., Baxter, G., Daniel-Issakani, S., and Strulovii, B. (1991) Proc. Natl. Acad. Sci. U.S.A. 88, 7988–7992
28. Wang, C., Constantinescu, S. N., MacEwan, D. J., Strulovii, B., Dekker, L. V., Parker, P. J., and Pfeffer, L. M. (1993) Proc. Natl. Acad. Sci. U.S.A. 90, 6944–6948
29. Hidaka, H., and Hagiwara, M. (1987) Trends Pharmacol. Sci. 8, 162–164
30. Koch, C. A., Anderson, D., Moran, M. F., Ellis, C., and Pawson, T. (1991) Science 252, 668–674
31. Pawson, T., and Schlessinger, J. (1993) Curr. Biol. 3, 434–442
32. Stahl, N., and Yanopoulos, G. D. (1993) Cell 74, 587–590
33. Wittnhoth, B. A., Quelle, F. W., Silvennoinen, O., Yi, T., Tang, B., Miura, O., and Ihle, J. N. (1993) Cell 74, 227–236
34. Songyang, Z., Shoetson, S. E., Chaudhuri, M., Gish, G., Pawson, T., Haser, W. G., King, F., Ratnasfky, S., Lechleider, R. J., Neil, B. G., Birge, R. G., Fajardo, J. E., Chou, M. M., Hananussa, H., Schaffhausen, B., and Cantley, L. C. (1993) Cell 72, 767–778
35. Eck, M. J., Atwell, S. K., Shoelston, S. E., and Harrison, S. C. (1994) Nature 368, 764–769
36. Panchamothy, G., Fukazawa, T., Sttlz, L., Payne, G., Reedquist, K., Shoetson, S., Songyang, Z., Cantley, L., Walsh, C., and Band, H. (1994) Mol. Cell. Biol. 14, 6372–6385
37. Greenlund, A. C., Farrar, M. A., Viviano, B. L., and Schreiber, R. D. (1994) EMBO J. 13, 1591–1600
38. Colamonic, O. R., Porterfield, B., Domanski, P., Constantinescu, S. N., and Pfeffer, L. M. (1994) J. Biol. Chem. 269, 9588–9602
39. Constantinescu, S. N., Croze, E., Murti, A., Wang, C., Basu, L., Hollander, D., Russell-Harde, D., Garcia-Martinez, V., Mullersman, J. E., and Pfeffer, L. M. (1995) Proc. Natl. Acad. Sci. U.S.A. 92, 10487–10491
40. Stahl, N., Farruggella, T. J., Boulton, T. G., Zhong, Z., Darnell, J. E., Jr., and Yanopoulos, G. D. (1995) Science 267, 1349–1353
Direct Association of STAT3 with the IFNAR-1 Chain of the Human Type I Interferon Receptor
Chuan-He Yang, Wei Shi, Leela Basu, Aruna Murti, Stefan N. Constantinescu, Lawrence Blatt, Ed Croze, Jerald E. Mullersman and Lawrence M. Pfeffer

J. Biol. Chem. 1996, 271:8057-8061.
doi: 10.1074/jbc.271.14.8057

Access the most updated version of this article at http://www.jbc.org/content/271/14/8057

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 40 references, 18 of which can be accessed free at http://www.jbc.org/content/271/14/8057.full.html#ref-list-1