Journal of Cellular Biochemistry 99:1078–1084 (2006)

JAK1 N-Terminus Binds to Conserved Box 1 and Box 2 Motifs of Cytokine Receptor Common β Subunit but Signal Activation Requires JAK1 C-Terminus

Huei-Mei Huang,* Ya-Li Lee, and Ting-Wei Chang

Graduate Institute of Cell and Molecular Biology, Taipei Medical University, Taipei, Taiwan

Abstract The human interleukin-3 receptor (hIL-3R) consists of a unique α subunit (hIL-3Rα) and a common β subunit (βc). Binding of IL-3 to IL-3R activates Janus kinases JAK1 and JAK2. Our previously study showed that JAK2 and JAK1 were constitutively associated with the hIL-3Rα and βc subunits, respectively. In this study, we further demonstrate that JAK2 binds to the intracellular domain of hIL-3Rα and JAK1 binds to the Box 1 and Box 2 motifs of βc using GST-hIL-3R fusion proteins in pull-down assays. JAK1 mutational analysis revealed that its JH7-3 domains bound directly to the Box 1 and Box 2 motifs of βc. We further examined the role of JAK1 JH7-3 domains in JAK1 and JAK2-mediated signaling using the CDJAKs fusion proteins, which consisted of a CD16 extracellular domain, a CD7 transmembrane domain, and either JAK1 (CDJAK1), JAK2 (CDJAK2), or JAK1-JH7-3 domains (CDJAK1-JH7-3) as intracellular domains. Anti-CD16 antibody crosslinking of wild type fusion proteins CDJAK1 with CDJAK2 could mimic IL-3 signaling, however, the crosslinking of fusion proteins CDJAK1-JH7-3 with CDJAK2 failed to activate downstream proteins. These results suggest that the JAK1-JH7-3 domains are required for βc interaction and abolish wild type JAK1 and JAK2-mediated signaling. J. Cell. Biochem. 99: 1078–1084, 2006.

Key words: JAK1; JAK2; JH domain; hIL-3Rα; βc; intracellular domain; Box 1 and Box 2 motifs; GST pull-down; association; fusion proteins

Cytokine receptors do not contain any intrinsic catalytic domains in their intracellular regions, they associate with and require cytoplasmic Janus kinases (JAKs) for signal transduction [Ihle et al., 1994]. JAKs (JAK1, JAK2, JAK3, and TYK2) are a family of non-receptor tyrosine kinases [Ihle and Kerr, 1995]. Different JAKs are associated with the various cytokine receptors, and can play different roles in individual receptor complexes. For example, JAK2 is the sole JAK activated in response to erythropoietin (EPO) [Witthuhn et al., 1993], whereas JAK1, JAK2, and TYK2 are activated in response to interleukin (IL)-6 [Guschin et al., 1995]. Seven JAK homology (JH) domains have been described [Ihle and Kerr, 1995], numbering from JH7 through JH1, from the N-terminus. The sequence of JH2 domain is similar to that of the JH1 kinase domain except for the lack of kinase activity. The JH2 domain has been implicated in regulating the kinase activity of the JH1 domain [Saharinen et al., 2000; Yeh et al., 2000]. In addition, JH2 domain associates with STATs [Fujitani et al., 1997]. There is much sequence variation within the N-terminal JH7-3 domains of the four members of the JAK family. The JH7-3 domains have been implicated in receptor association [Kisseleva et al., 2002] and in controlling the JAK kinase activity [Zhou et al., 2001]; this implies that these JH domains could be involved in signal transduction.

The cytokine IL-3 regulates proliferation, differentiation, and anti-apoptosis of hematopoietic cells [Arai et al., 1990; Crompton, 1991]. The human receptor for IL-3 consists of an IL-3-specific α subunit (IL-3Rα) and a β subunit (βc)

Grant sponsor: National Science Council (Taiwan); Grant number: NSC 91-2320-B-038-028.

*Correspondence to: Huei-Mei Huang, Graduate Institute of Cell and Molecular Biology, Taipei Medical University, No. 250, Wu-Hsing Street, Hsinyi District, Taipei 110, Taiwan. E-mail: cmbhhm@tmu.edu.tw

Received 15 February 2006; Accepted 8 March 2006

DOI 10.1002/jcb.20942

that is in common with IL-5 and granulocyte-macrophage colony-stimulating factor (GM-CSF) receptors [Miyajima et al., 1993; Orban et al., 1999]. In the mouse, IL-3 and IL-3Rα can form functional IL-3 receptor complexes by interacting with either of two type of highly homologous β subunits, βc and $\beta_{IL\text{-}3}$ (the IL-3-specific β-chain). $\beta_{IL\text{-}3}$ is the product of a relatively recent duplication of βc [Itoh et al., 1990; Hara and Miyajima, 1992]. Binding of all three cytokines to their respective receptors induce the activation of JAK1 and JAK2 [Ogata et al., 1998; Huang et al., 2000, 2005]. The intracellular domain of βc, particularly the membrane-proximal region containing the Box 1 motif, has been shown to regulate JAK2 activation in the GM-CSFR system [Watanabe et al., 1996]. It has been shown that JAK2 and JAK1 constitutively associate with the IL-5Rα and βc subunits, respectively [Ogata et al., 1998]. We recently reported that JAK2 and JAK1 pre-associated with the IL-3Rα and βc subunits, respectively, prior to activation in vivo [Huang et al., 2005]. In the mechanism of JAK1 and JAK2-mediated signaling in IL-3 system, we suggest that a large functional complex of JAK2-IL-3Rα and JAK1-β is formed after IL-3 binds to IL-3R, and then JAK1 and JAK2 within this functional complex undergo tyrosine phosphorylation. The simultaneous activation of both JAK1 and JAK2 trigger further downstream signaling [Huang et al., 2005]. The understanding of human IL-3, IL-5, and GM-CSF signaling will require clear definition of the structure/function relationships of JAK1/βc complexes. At present, the nature of the interacting domain(s) between JAK1 and βc remains unclear.

In this study, we characterized the protein domains of JAK1 and βc responsible for association. Furthermore, we ask if the JAK1 domains for βc association inhibit JAK1 and JAK2-mediated signaling pathways.

MATERIALS AND METHODS

Cell Lines and Materials

Ba/F3 cells (murine IL-3-dependent pro-B cells) were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1%-conditioned medium from WEHI-3B cells as a source of IL-3. For cytokine stimulation experiments, cells were starved in 0.5% FBS medium for 12 h before being stimulated by 10 ng/ml IL-3 (R&D Systems, Minneapolis, MN). Ba/F3-CDJ1 + 2 clones stably coexpressed CDJAK1 and CDJAK2 in Ba/F3 cells, and Ba/F3-CDJ2 clones stably expressed CDJAK2 in Ba/F3 cells as described previously [Huang et al., 2005]. Anti-human CD16 $F(ab')_2$ antibody was purchased from Ancell Corporation (Bayport, MN). Antibodies specific for JAK1, JAK2, and STAT5 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Antibodies specific for phospho-ERK1/2, ERK1/2, phospho-Akt and Akt were purchased from New England Biolabs, Inc. (Beverly, MA). The anti-phosphotyrosine antibody (4G10) was obtained from Upstate Biotechnology (Lake Placid, NY). Monoclonal anti-α tubulin antibody was purchased from Amersham Pharmacia Biotech, Inc. (San Francisco, CA).

GST Fusion Proteins

The coding sequences for the intracellular domain (ICD) of human IL-3Rα was amplified by polymerase chain reaction (PCR) and subcloned into pGEX2T (Amersham Biosciences) in-frame with the glutathione S-transferase (GST) coding sequence (GST-IL-3RαICD). Plasmid encoding GST fusion protein with the βc Box 1 and Box 2 motifs (GST-βcBox 1 + 2) was constructed by ligating the βc Box 1 and Box 2 fragment into pGEX1 vector. The βc Box 1 and Box 2 fragment was prepared by *Fsp*I/*Pvu*II restriction of βc cDNA followed by a fill-in reaction, and corresponds to amino acid sequence 472 to 557 of βc. Constructs were verified by sequencing. These GST fusion proteins were expressed in *Escherichia coli*, and were affinity-purified on glutathione-Sepharose (Amersham Biosciences).

For GST pull-down assay, equal amounts of GST fusion proteins were incubated with JAK1 and JAK2 lysates from in vitro transcription/translation assays. After adding sample buffer, the precipitates bound to GST fusion proteins were eluted by boiling, separated by SDS–PAGE, and imaged by autoradiography.

JAKs Constructs and In Vitro Transcription/Translation Assays

The plasmids encoding full-length JAK1 and JAK2 have been previously described [Huang et al., 2005]. JAK1-JH6-1 and JAK1-JH5-1 were produced by replacing full-length JAK1 with JH6-3 and JH5-3 PCR fragments, respectively (JH6-3 represents JH6 to JH3 domains of JAK1,

inclusive, other constructs are similarly represented). JAK1-JH7-3 was generated by deletion of a *Afl*II/*Eco*RI fragment encoding the JH2-1 domains. JAK1-JH7-6 was generated by deletion of a *Bam*HI fragment encoding the JH5-1 domains. The coding sequences for JH4-3 was amplified by PCR and subcloned into pBluescriptKS (JAK1-JH4-3). Plasmid pCD16/CD7/JAK1-JH7-3 was constructed by ligating the JH7-3 fragment, a *Mlu*I/*Not*I restriction fragment of the JAK1-JH7-3 plasmid, and subcloning it into pEFCD16/CD7 [Huang et al., 2005]. In vitro transcription/translation assays were performed using a commercial kit (Promega, Madison, WI).

Establishment of Ba/F3-CDJ1(JH7-3) + 2 Cells

Ba/F3-CDJ2 cells were transfected with the plasmid pCD16/CD7/JAK1-JH7-3 by electroporation at 300 V, 40 kHz using a BioRad apparatus. Stable transfectants were selected with hygromycin B.

Antibody Crosslinking and Western Blot Analysis

Antibody crosslinking was performed as described previously [Huang et al., 2005]. Briefly, cells were starved for 12 h in RPMI-1640 medium supplemented with 0.5% FBS. Cells (10^7) were resuspended per 0.5 ml of original medium and incubated with or without (control) 1 μg of anti-CD16 $F(ab')_2$ antibody for 5 min at room temperature, followed by incubation with 5 μg/ml of anti-mouse IgG $F(ab')_2$ for 15 min at 37°C. For Western blot analysis, cells were lysed in lysis buffer (1% Triton X-100, 20 mM Tris pH 7.5, 150 mM NaCl, 1 mM EGTA, 1 mM EDTA, 2.5 mM sodium pyrophosphate, 1mM β-glycerolphosphate, 1 mM PMSF, 1 mg/ml leupeptin, and 1 mM Na_3VO_4). Immunoprecipitation and Western blotting were performed as described previously [Huang et al., 2005].

RESULTS

JAK2 Associated With hIL-3RαICD and JAK1 Associated With Box 1 and Box 2 Motifs of βc, In Vitro

Previously we showed that JAK2 and JAK1 were pre-associated with the IL-3Rα and βc subunits, respectively, in vivo [Huang et al., 2005]. To further determine the role of the intracellular domain of hIL-3R in JAK1 and JAK2 association, we generated two GST fusion proteins containing the entire intracellular domain (GST-IL3RαICD) and the Box 1 and Box 2 motifs of βc (GST-βcBox 1 + 2) (Fig. 1A). We performed binding assays to determine the association of JAKs (JAK1 and JAK2) with these GST fusion proteins. The JAKs are known to interact with two short motifs (Box 1 and Box 2) in the intracellular, membrane proximal region of cytokine receptors; such receptors include the gp130 subunit of the IL-6 family receptors [Murakami et al., 1991; Tanner et al., 1995], the EPO receptor [Witthuhn et al., 1993], the prolactin receptors [DaSilva et al., 1994], and the type I interferon receptor [Usacheva et al., 2002a]. Thus, we examined whether these regions of βc are critical for JAK1 binding. The GST pull-down assays displayed the direct binding of JAK2 to the IL-3RαICD (Fig. 1B) and the direct binding of JAK1 to the Box 1 and Box 2 motifs of βc (Fig. 1C). However, JAK1 and JAK2 did not associate with GST controls.

N-Terminal JH7-3 Domains of JAK1 Are Necessary for Association With Box 1 and Box 2 Motifs of βc

To map the βcBox 1 + 2-binding region of JAK1, we constructed JAK1 mutations with deletions of the following domains: the

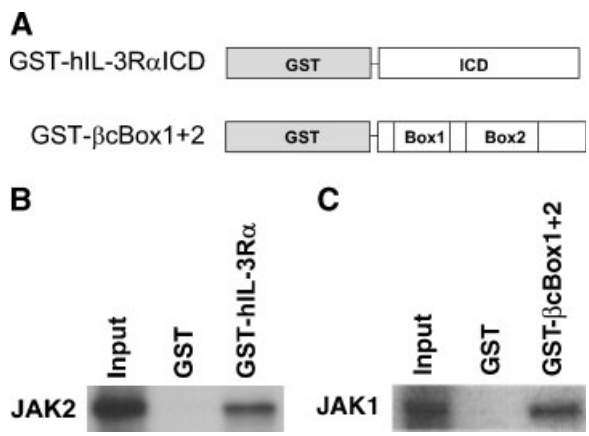

Fig. 1. Binding of JAK1 and JAK2 to distinct IL-3 receptor subunits. **A**: Schematic representation of the different GST fusion proteins used to study the association with JAK1 and JAK2. GST-IL-3Rα protein encodes the entire intracellular domain of IL-3Rα. GST-βcBox 1 + 2 protein encodes the Box 1 and Box 2 motifs of the βc intracellular domain. **B** and **C**: The GST-IL-3Rα and GST-βcBox 1 + 2 fusion proteins were used to pull down JAK2 and JAK1, respectively. JAK2 and JAK1 proteins were produced by in vitro transcription/translation assays. Pull-downs with GST alone were used as negative controls; the [^{35}S]methionine-labeled proteins, input, was the positive control.

JH7 domain (JAK1-JH6-1), JH7-6 domains (JAK1-JH5-1), JH7-5 and JH2-1 domains (JAK1-JH4-3), JH2-1 domains (JAK1-JH7-3), or JH5-1 domains (JAK1-JH7-6) (Fig. 2A). The [^{35}S]methionine-labeled JAK1 mutant proteins used for pull-down experiments were produced using an in vitro transcription/translation system. Figure 2B shows that the GST fusion proteins encoding the Box 1 and Box 2 motifs of βc associated with the full-length JAK1 and with the mutant kinase with a deletion of JH2-1 domains, but very weakly with JAK1-JH6-1, JAK1-JH5-1, JAK1-JH4-3, or JAK1-JH7-6. These results suggest that the JH7-3 domains of JAK1 participated in the binding with the Box 1 and Box 2 motifs of βc.

JH7-3 Domains of JAK1 Abolished the JAK1 and JAK2-Mediated Signaling Pathways

To determine the biological role of the JH7-3 domains of JAK1 in JAK1 and JAK2-mediated signaling, we generated a chimeric transmembrane protein containing the extracellular domain of CD16, the transmembrane domains of CD7, and the JH7-3 domains of JAK1 (CDJAK1-JH7-3). The CDJAK1-JH7-3 was stably expressed in Ba/F3 clones that also overexpressed CDJAK2 [Huang et al., 2005]; this clone was named Ba/F3-CDJ1(JH7-3) + 2 cells.

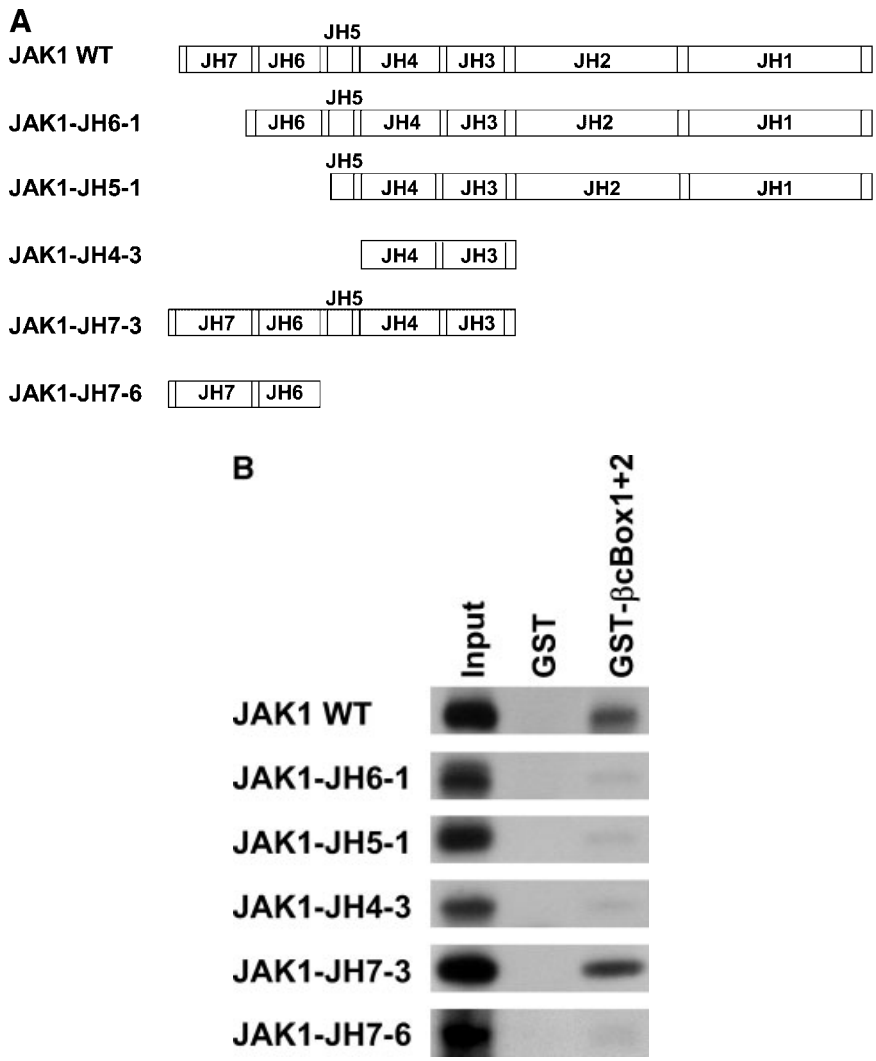

Fig. 2. The JH7-3 domains of JAK1 mediated the association with βc. **A**: Schematic representation of the different JAK1 mutants used to map the JAK1 binding site with βcBox 1 + 2. **B**: JAK1 wild type (WT) or the indicated mutant proteins were produced by in vitro transcription/translation then used for pull-down experiments with the GST-βcBox 1 + 2 fusion proteins. Pull-downs with GST alone were used as negative controls; the [^{35}S]methionine-labeled proteins, input, was the positive control.

Western blotting (Fig. 3) shows that the expression level of CDJAK1-JH7-3 fusion proteins in Ba/F3-CDJ1(JH7-3) + 2 cells were similar to that of CDJAK1 fusion proteins in Ba/F3-CDJ1 + 2 cells [Huang et al., 2005]. The CDJAK1-JH7-3 fusion proteins were the same molecular size as the endogenous JAK1 (Fig. 3). Previously, we demonstrated that the simultaneous activation of both CDJAK1 and CDJAK2 fusion proteins with antibody crosslinking led to the activation of signaling proteins [Huang et al., 2005]. The CDJAK1 and CDJAK2 were also pre-associated with the βc and IL-3Rα subunits, respectively [Huang et al., 2005]. Hence, we examined whether the JH7-3 domains of JAK1 interfere with JAK1 and JAK2-mediated signaling after antibody crosslinking in Ba/F3-CDJ1(JH7-3) + 2 cells. Phosphorylation signals of downstream proteins were examined by Western blotting using anti-phospho-Akt and anti-phospho-MAPK antibodies. The Western blots were stripped and reprobed with anti-Akt, or anti-MAPK antibodies as internal controls. We also assessed the activation of STAT5 as a downstream effector of JAK1 and JAK2 by anti-STAT5 antibody immunoprecipitation followed by Western blotting using phosphotyrosine antibody, then reprobed with anti-STAT5 antibody. Figure 4 shows that crosslinking of CDJAK1-JH7-3 and CDJAK2 fusion proteins failed to induce tyrosine phosphorylation of Akt, MAPK and STAT5 in the absence of IL-3 in Ba/F3-CDJ1(JH7-3) + 2 cells. However, the addition of IL-3 or the crosslinking of CDJAK1 and CDJAK2 fusion proteins both induced very high levels of tyrosine phosphorylation of the same downstream proteins in parental Ba/F3 cells and in Ba/F3-CDJ1 + 2 cells, respectively (Fig. 4).

DISCUSSION

Ligand-induced dimerization of receptor subunits causes JAKs activation and triggers a series of downstream signaling events that induce transcription of different genes responsible for the physiological effects of the specific cytokine. Similar results have been reported for the association of tyrosine kinase JAK1 with βc, an important event for IL-3 receptor to exert its function [Huang et al., 2005]. Therefore, it is critical to define the domains in JAK1 and βc responsible for this association to mimic or

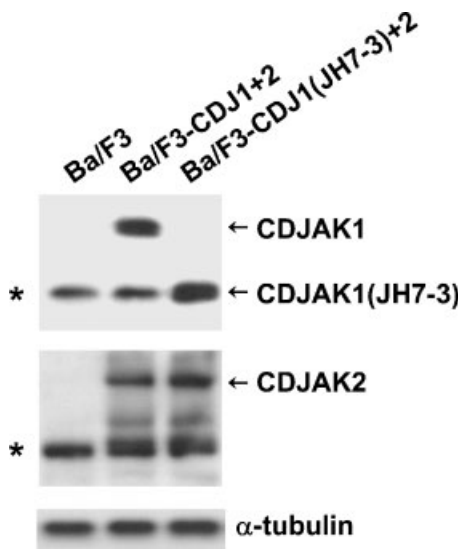

Fig. 3. Expression of CDJAK1-JH7-3 and CDJAK2 proteins in Ba/F3-CDJ1(JH7-3) + 2 cells. Protein expression in Ba/F3, Ba/F3-CDJ1 + 2 and Ba/F3-CDJ1(JH7-3) + 2 cells were confirmed using SDS–PAGE followed by Western blotting with anti-JAK1 antibody, and reprobed with anti-JAK2 antibody. The membrane was reprobed with anti-α tubulin antibody as internal control. Arrows indicate CDJAK fusion proteins while asterisks indicate endogenous JAKs.

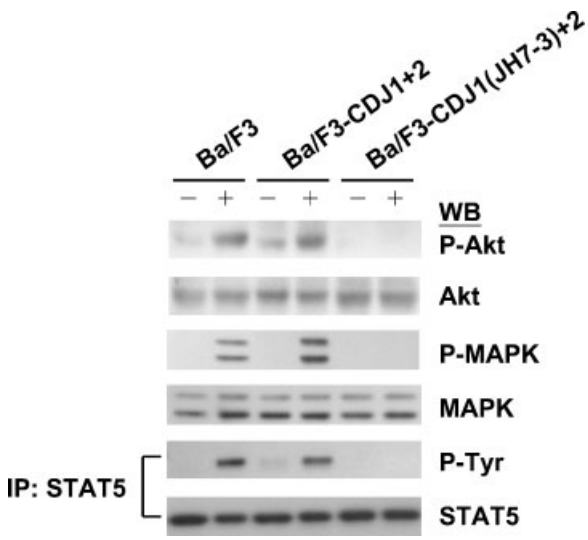

Fig. 4. Crosslinking of CDJAK1-JH7-3 and CDJAK2 abolished phosphorylation of Akt, MAPK, and STAT5 in Ba/F3-CDJ1(JH7-3) + 2 cells. Starved Ba/F3-derived transfectants were crosslinked with or without antibody. Cell lysates were prepared and resolved by SDS–PAGE, Western blotted (WB) with anti-phospho-Akt (P-Akt) or anti-phospho-MAPK (P-MAPK) antibody, then reprobed with anti-Akt or anti-MAPK antibody. For STAT5 analysis, cell lysates were immunoprecipitated (IP) with anti-STAT5 antibody and Western blotted with anti-phosphotyrosine (P-tyr) antibody, then reprobed with anti-STAT5 antibody. Starved Ba/F3 cells were stimulated with or without IL-3 as controls.

abolish the activation of cytokine systems. Some reports have indicated that the N-terminal region, containing the JH7-6 domains of JAK1, JAK2, JAK3, and TYK2 are critical for the association with receptors [Frank et al., 1995; Chen et al., 1997; Gauzzi et al., 1997; Richter et al., 1998; Cacalano et al., 1999; Usacheva et al., 2002b]. In addition, the JH6-3 domains of JAK1 are necessary for the association with cytokine receptors [Usacheva et al., 2002b]. Our data indicate that the JH7-3 domains of JAK1 associated with the Box 1 and Box 2 motifs of βc. These data suggest that JAK1 does not associate in the same manner with the other cytokine receptors.

Structural modeling has determined that JH4-3 domains of JAK1 share some similarity with SH2 domains, and that the JH7-4 domains constitute a FERM (four-point-one, Ezrin, Radixin, Moesin) homology domain [Girault et al., 1998]. Recently, report showed that the JAK1 region encompassing the FERM domain may be critical for functional binding to the intracellular domain of gp130 [Hilkens et al., 2001]. Hence, finer mapping is required to determine if the JH7-3 domains of JAK1 for binding to βc are through the FERM domain. Finally, we examined the role of JAK1 JH7-3 domains in JAK1 and JAK2-mediated signaling using the CDJAKs chimeric transmembrane proteins. Crosslinking of CDJAK1 and CDJAK2 fusion proteins (using wild type JAKs) could mimic IL-3 signaling [Huang et al., 2005; Fig. 4]. However, crosslinking of CDJAK1-JH7-3 and CDJAK2 fusion proteins failed to activate Akt, MAPK nor STAT5. Thus, the deletion of JAK1 kinase domain (JH2-1 domains) abolished JAK1 and JAK2-mediated signaling, similar to our recent report that the CD fusion protein containing JAK1 dominant negative mutant (CDJAK1KE) crosslinked with CDJAK2 inhibited JAK1 and JAK2-mediated signaling [Huang et al., 2005].

The Box 1 and Box 2 motifs proximal to the membrane region of cytokine receptors are known to associate with JAK. In our studies, the Box 1 and Box 2 motifs of βc bound JAK2 in in vitro studies (Figs. 1C and 2B); this is consistent with that seen in the other receptors, including the gp130 subunit of the IL-6 family receptors [Murakami et al., 1991; Tanner et al., 1995], the EPO receptor [Witthuhn et al., 1993], the prolactin receptors [DaSilva et al., 1994], and the type I interferon receptor [Usacheva et al., 2002a]. These data suggest that the Box 1 and Box 2 motifs of cytokine receptors are conserved regions for association with JAK. The deletion mutation of hIL-5Rα in the proline-rich region of intracellular domain was unable to bind JAK2 [Ogata et al., 1998]. Whether the proline-rich region of hIL-3Rα intracellular domain associate with JAK2 needs to be proven.

JAK2 pre-association with IL-3RαICD and JAK1 pre-association with the Box 1 and Box 2 motifs of βc are consistent with that seen in the IL-5 system, in which JAK2 was shown to constitutively associate with IL-5Rα and JAK1 was shown to constitutively associate with βc [Ogata et al., 1998]. However, other studies have reported ligand-induced JAK2 binding to the β subunit in the IL-3 or GM-CSF system [Brizzi et al., 1996; Cattaneo et al., 1996; Chin et al., 1997] as discussed previously [Ogata et al., 1998; Huang et al., 2005].

In summary, our data indicate that direct associations between JAKs (JAK1 and JAK2) and IL-3R subunits were identified. This report provides the first evidence that the N-terminal JH7-3 domains of JAK1 are necessary for association with the Box 1 and Box 2 motifs of βc, and this βc binding domain of JAK1 inhibits signaling. Although the exact sequences within this interacting region of JAK1 have yet to be determined, our findings have delineated a restricted domain that is essential for JAK1-βc interaction.

REFERENCES

Arai KI, Lee F, Miyajima A, Miyatake S, Arai N, Yokota T. 1990. Cytokines: Coordinators of immune and inflammatory responses. Annu Rev Biochem 59:783–836.

Brizzi MF, Aronica MG, Rosso A, Bagnara GP, Yarden Y, Pegoraro L. 1996. Granulocyte-macrophage colony-stimulating factor stimulates JAK2 signaling pathway and rapidly activates p93fes, STAT1 p91, and STAT3 p92 in polymorphonuclear leukocytes. J Biol Chem 271:3562–3567.

Cacalano N, Migone TS, Bazan F, Hanson EP, Chen M, Candotti F, O'Shea JJ, Johnston JA. 1999. Autosomal SCID caused by a point mutation in the N-terminus of Jak3: Mapping of the Jak3-receptor interaction domain. EMBO J 18:1549–1558.

Cattaneo E, De Fraja C, Conti L, Reinach B, Bolis L, Govoni /SNM> S, Liboi E. 1996. Activation of the JAK/STAT pathway leads to proliferation of ST14A central nervous system progenitor cells. J Biol Chem 271:23374–23379.

Chen M, Cheng A, Chen YQ, Hymel A, Hanson EP, Kimmel L, Minami Y, Taniguchi T, Changelian PS, O'Shea JJ.

1997. The amino terminus of JAK3 is necessary and sufficient for binding to the common chain and confers the ability to transmit interleukin 2-mediated signals. Proc Natl Acad Sci USA 94:6910–6915.

Chin H, Wakao H, Miyajima A, Kamiyama R, Miyasaka N, Miura O. 1997. Erythropoietin induces tyrosine phosphorylation of the interleukin-3 receptor beta subunit (betaIL3) and recruitment of Stat5 to possible Stat5-docking sites in betaIL3. Blood 89:4327–4336.

Crompton T. 1991. IL3-dependent cells die by apoptosis on removal of their growth factor. Growth Factors 4:109–116.

DaSilva L, Howard OM, Rui H, Kirken RA, Farrar WL. 1994. Growth signaling and JAK2 association mediated by membrane-proximal cytoplasmic regions of prolactin receptors. J Biol Chem 269:18267–18270.

Frank SJ, Yi W, Gilliland G, Jiang J, Sakai I, Kraft AS. 1995. Regions of the Jak2 tyrosine kinase required for coupling to the growth hormone receptor. J Biol Chem 270:14776–14785.

Fujitani Y, Hibi M, Fukada T, Takahashi-Tezuka M, Yoshida H, Yamaguchi T, Sugiyama K, Yamanaka Y, Nakajima K, Hirano T. 1997. An alternative pathway for STAT activation that is mediated by the direct interaction between JAK and STAT. Oncogene 14:751–761.

Gauzzi MC, Barbieri G, Richter MF, Uze G, Ling L, Fellous M, Pellegrini S. 1997. The amino-terminal region of Tyk2 sustains the level of interferon alpha receptor 1, a component of the interferon alpha/beta receptor. Proc Natl Acad Sci USA 94:11839–11844.

Girault JA, Labesse G, Mornon JP, Callebaut I. 1998. Janus kinases and focal adhesion kinases play in the 4.1 band: A superfamily of band 4.1 domains important for cell structure and signal transduction. Mol Med 4:751–769.

Guschin D, Rogers N, Briscoe J, Witthuhn B., Watling D, Horn F, Pellegrini S, Yasukawa K, Heinrich P, Stark GR, Ihle JN, Kerr IM. 1995. A major role for the protein tyrosine kinase JAK1 in the JAK/STAT signal transduction pathway in response to interleukin-6. EMBO J 14:1421–1429.

Hilkens CM, Is'harc H, Lillemeier BF, Strobl B, Bates PA, Behrmann I, Kerr IM. 2001. A region encompassing the FERM domain of Jak1 is necessary for binding to the cytokine receptor gp130. FEBS Lett 505:87–91.

Huang HM, Huang CJ, Yen JJ. 2000. Mcl-1 is a common target of stem cell factor and interleukin-5 for apoptosis prevention activity via MEK/MAPK and PI-3K/Akt pathways. Blood 96:1764–1771.

Huang HM, Lin YL, Chen CH, Chang TW. 2005. Simultaneous activation of JAK1 and JAK2 confers IL-3 independent growth on Ba/F3 pro-B cells. J Cell Biochem 96:361–375.

Ihle JN, Kerr IM. 1995. Jaks and Stats in signaling by the cytokine receptor superfamily. Trends Genet 11: 69–74.

Ihle JN, Witthuhn BA, Quelle FW, Yamamoto K, Thierfelder WE, Kreider B, Silvennoinen O. 1994. Signaling by the cytokine receptor superfamily: JAKs and STATs. Trends Biochem Sci 19:222–227.

Itoh N, Yonehara S, Schreurs J, Gorman DM, Maruyama K, Ishii A, Yahara I, Arai K, Miyajima A. 1990. Cloning of an interleukin-3 receptor gene: a member of a distinct receptor gene family. Science 247:324–327.

Kisseleva T, Bhattacharya S, Braunstein J, Schindler CW. 2002. Signaling through the JAK/STAT pathway, recent advances and future challenges. Gene 285:1–24.

Miyajima A, Mui AL, Ogorochi T, Sakamaki K. 1993. Receptors for granulocyte-macrophage colony-stimulating factor, interleukin-3, and interleukin-5. Blood 82: 1960–1974.

Murakami M, Narazaki M, Hibi M, Yawata H, Yasukawa K, Hamaguchi M, Taga T, Kishimoto T. 1991. Critical cytoplasmic region of the interleukin 6 signal transducer gp130 is conserved in the cytokine receptor family. Proc Natl Acad Sci USA 88:11349–11353.

Ogata N, Kouro T, Yamada A, Koike M, Hanai N, Ishikawa T, Takatsu K. 1998. JAK2 and JAK1 constitutively associate with an interleukin-5 (IL-5) receptor alpha and betac subunit, respectively, and are activated upon IL-5 stimulation. Blood 91:2264–2271.

Orban PC, Levings MK, Schrader JW. 1999. Heterodimerization of the alpha and beta chains of the interleukin-3 (IL-3) receptor is necessary and sufficient for IL-3-induced mitogenesis. Blood 94:1614–1622.

Richter MF, Dumehnil G, Uze G, Fellous M, Pellegrini S. 1998. Specific contribution of Tyk2 JH regions to the binding and the expression of the interferon alpha/beta receptor component IFNAR1. J Biol Chem 273:24723–24729.

Saharinen P, Takaluoma K, Silvennoinen O. 2000. Regulation of the Jak2 tyrosine kinase by its pseudokinase domain. Mol Cell Biol 20:3387–3395.

Tanner JW, Chen W, Young RL, Longmore GD, Shaw AS. 1995. The conserved box 1 motif of cytokine receptors is required for association with JAK kinases. J Biol Chem 270:6523–6530.

Usacheva A, Sandoval R, Domanski P, Kotenko SV, Nelms K, Goldsmith MA, Colamonici OR. 2002a. Contribution of the Box 1 and Box 2 motifs of cytokine receptors to Jak1 association and activation. J Biol Chem 277:48220–48226.

Usacheva A, Kotenko S, Witte MM, Colamonici OR. 2002b. Two distinct domains within the N-terminal region of Janus kinase 1 interact with cytokine receptors. J Immunol 169:1302–1308.

Watanabe S, Itoh T, Arai K. 1996. JAK2 is essential for activation of c-*fos* and c-*myc* promoters and cell proliferation through the human granulocyte-macrophage colony-stimulating factor receptors in BA/F3 cells. J Biol Chem 271:12681–12682.

Witthuhn BA, Quelle FW, Silvennoinen O, Yi T, Tang B, Miura O, Ihle JN. 1993. JAK2 associates with the erythropoietin receptor and is tyrosine phosphorylated and activated following stimulation with erythropoietin. Cell 74:227–236.

Yeh TC, Dondi E, Uze G, Pellegrini S. 2000. A dual role for the kinase-like domain of the tyrosine kinase Tyk2 in interferon-alpha signaling. Proc Natl Acad Sci USA 97:8991–8996.

Zhou YJ, Chen M, Cusack NA, Kimmel LH, Magnuson KS, Boyd JG, Lin W, Roberts JL, Lengi A, Buckley RH, Geahlen RL, Candotti F, Gadina M, Changelian PS, O'Shea JJ. 2001. Unexpected effects of FERM domain mutations on catalytic activity of Jak3: Structural implication for Janus kinases. Mol Cell 8:959–969.