The Janus Kinase Family and Signaling Through Members of the Cytokine Receptor Superfamily (43757)

JAMES N. IHLE[1]

Department of Biochemistry, St. Jude Children's Research Hospital, Memphis, Tennessee 38105

Abstract. **Many cytokines initiate cellular responses through their interaction with members of the cytokine receptor superfamily which contain no catalytic domains in their cytoplasmic domains. Irrespective, ligand binding induces tyrosine phosphorylation, which requires a membrane proximal region of the cytoplasmic domain. Recent studies have shown that members of the Janus kinase (JAK) family of protein tyrosine kinases associate with the membrane proximal region, are rapidly tyrosine phosphorylated following ligand binding and their *in vitro* kinase activity is activated. The JAKs are 130-kDa proteins which lack SH2/SH3 domains and contain two kinase domains, an active domain and a second kinase-like domain. Individual receptors associate with, or require, one or more of the three known family members including JAK1, JAK2, and tyk2. Substrates of the JAKs include the 91-kDa and 113-kDa proteins of the interferon-stimulated transcription complex ISGF3. These proteins, when tyrosine phosphorylated, migrate to the nucleus and participate in the activation of gene transcription. Recent evidence suggests that the 91- and 113-kDa proteins are members of a large family of genes that are potential substrates of JAK family members and may regulate a variety of genes involved in cell growth, differentiation or function.**

[P.S.E.B.M. 1994, Vol 206]

The Cytokine Receptor Superfamily

The growth, differentiation, and functional activities of cells are regulated through the interaction of growth factors or cytokines with their cognate receptors (1, 2). A number of growth factors act through receptor protein tyrosine kinases and mediate a biological response by activation of their intrinsic protein kinase activity (3). A large number of growth factors or cytokines, however, bind receptors of the cytokine receptor superfamily (4). These factors affect many lineages of cells and include the interleukins (IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, and IL-13), colony-stimulating factors (G-CSF, GM-CSF), ciliary neurotropic factor (CNTF), leukemia inhibitory factor (LIF), erythropoietin (Epo), prolactin, oncostatin M (OSM), growth hormone (GH) and the interferons (IFN-α/β, IFN-γ). The type I receptors share conserved motifs in the extracellular domains consisting of four positionally conserved cysteines and the WSXWS motif. Receptors of the cytokine receptor superfamily have only limited similarity in their cytoplasmic domains. In spite of this, a membrane proximal region of the cytoplasmic domain of one or more of the receptor subunits is required for mitogenesis termed the box1 and box2 motifs (5).

In spite of the lack of a kinase domain, receptors of the cytokine superfamily rapidly induce tyrosine phosphorylation of cellular substrates as well as the receptors. In those cases examined, the membrane proximal domain is required for tyrosine phosphorylation. The rapid induction of tyrosine phosphorylation, the phosphorylation of the receptors, and the detection of kinase activity in receptor immunoprecipitates have all led to the hypothesis that a protein tyrosine kinase physically associates with the membrane proximal region of cytokine receptors and becomes activated following ligand binding.

Several studies have focused on the potential role of members of the *src* gene family. Initially, it was demonstrated that *lck* associates with the IL-2 recep-

[1] To whom requests for reprints should be addressed at Department of Biochemistry, St. Jude Children's Research Hospital, 332 North Lauderdale, Memphis, TN 38105.

0037-9727/94/2063-0268$10.50/0

tor β chain; however, the binding and activation of *lck* do not correlate with the mitogenic activity of mutant receptors (6). Subsequent studies have also implicated the related kinases, *fyn* and *lyn*, in IL-2 (7) and IL-3 signaling (8). More recently, the *fes* kinase has been implicated in signaling through the receptors for IL-3 and for erythropoietin (9, 10).

The Janus Kinase Family of Tyrosine Kinases

Compelling evidence that the Janus kinase (JAK) families play a central role in the signaling of the cytokine receptor superfamily has appeared over the past year. The JAK kinases were first detected in studies designed to identify novel protein tyrosine kinases by a polymerase chain reaction (PCR) approach (11, 12). This approach identified a highly represented, novel kinase domain, and efforts were initiated to obtain full-length cDNAs. Subsequently full-length cDNAs have been obtained for murine JAK1 (Silvennoinen *et al.*, submitted[2]), murine JAK2 (13) and human JAK1 (14). The term JAK was proposed as an acronym for Janus kinase. Janus is an ancient Roman god of gates and doorways, depicted with two faces looking in opposite directions. Because, at the time of their isolation, a variety of novel protein tyrosine kinases were being identified through comparable strategies, the term JAK was also, affectionately, used to stand for *just another kinase*. At about the same time, screening of a human lymphoid library under low stringency conditions with a c-*fms* probe allowed the isolation of cDNAs for another novel kinase termed tyrosine kinase 2, tyk2 (15). Characterization of full-length cDNAs identified considerable similarity between the two kinases identified by PCR amplification (JAK1, JAK2) and tyk2, suggesting that these three kinases constituted a unique subfamily of protein tyrosine kinases.

The most striking feature of the family is the presence of two kinase domains. The more carboxyl domain contains all the consensus sequences that are associated with tyrosine kinases (16). Immediately amino-terminal is a second kinase-like domain which lacks several residues that are essential for kinase activity. Therefore, it would be predicted that the carboxyl domain encodes an active tyrosine kinase, while it is unlikely that the amino-terminal domain would have any kinase activity.

The sequences amino-terminal to the kinase domains bear no homology with previously described protein motifs including SH2 or SH3 domains that are critical for the function of a variety of cytoplasmic protein tyrosine kinases (17). Although lacking known motifs, the amino-terminal regions of the JAK kinases share considerable similarity and allow the identification of JAK homology domains. It can be hypothesized that the conserved regions involved in the association of the JAK kinases were various cytokine receptors.

The JAK Kinases in Cytokine Signaling

The first indication that JAK kinases might be involved in cytokine signaling came from studies with a mutant cell line (UI) that had lost the ability to respond to IFN-α/β (18). By transfection approaches, a human genomic fragment was isolated which was capable of restoring the IFN response of the mutant. This fragment was found to encode the human tyk2 gene.

The full importance of the JAK kinases in cytokine signaling however, became more apparent with the demonstration that JAK2 was involved in the signaling pathways for Epo (19), IL-3 (13), and GH (20). Studies with the Epo receptor had demonstrated that a membrane proximal region of the cytoplasmic domain of the receptor was essential for mitogenesis and for coupling ligand binding to the induction of tyrosine phosphorylation (21). Initially, the ability of Epo to induce tyrosine phosphorylation of the various kinases was examined. Strikingly, Epo dramatically induced tyrosine phosphorylation of JAK2 while having no effect on any of the other kinases (19). Epo also induced activation of the *in vitro* kinase activity of JAK2. Lastly, it was shown that Epo physically associates with the membrane proximal region of the cytoplasmic domain of the Epo receptor.

The importance of JAK2 activation in Epo signaling was indicated by examining a series of mutations of the receptor and finding a complete correlation between the loss or retention of the ability to support mitogenesis and the activation of JAK2. In particular, carboxyl truncations of the receptor that did not affect mitogenesis similarly did not affect JAK2 association or activation. Conversely, deletions or mutations in the membrane proximal region of the cytoplasmic domain of the receptor that inactivated the receptor for mitogenesis were also ineffective in JAK2 activation. GH was also found to rapidly induce tyrosine phosphorylation of JAK2 and to activate its *in vitro* kinase activity, comparable to the results obtained with Epo (20). More recently comparable studies have been done with prolactin (22). Similar to Epo and GH, prolactin stimulation of a prolactin responsive cell line, Nb_2, or mouse mammary gland explants, induced tyrosine phosphorylation of JAK2 and activation of its *in vitro* kinase activity. Similar to the receptor for Epo, JAK2 is constitutively associated with the prolactin receptor.

The receptors for GH, prolactin, and Epo consist of single chains. In contrast, the receptor for IL-3 is

[2] Silvennoinen O, Copeland N, Jenkins NA, Ihle JN. Structure of the murine JAK1 and mapping of murine JAK1 and JAK2 to chromosomes 4 and 19 respectively. (Submitted 1993).

composed of α and β subunits, both of which are members of the cytokine receptor superfamily (23). The β subunit is also utilized in the formation of the high-affinity receptors for GM-CSF and IL-5. IL-3 rapidly induces the tyrosine phosphorylation of JAK2 and activates its *in vitro* kinase activity (13) comparable to GH and Epo. The activation of JAK2 requires the membrane proximal domain of the β subunit as well as the cytoplasmic domain of the α subunit (Quelle F, Ihle JN, unpublished data). Importantly, the membrane proximal domain of the β chain had been previously shown to be required for induction of a mitogenic response but was not required for coupling of ligand binding to activation of components of the Ras signaling pathway (24).

More recently, JAK2 has also been implicated in the signaling pathway utilized by G-CSF (Witthuhn B and Ihle JN, unpublished data). The G-CSF receptor consists of a single chain which can mediate quite distinct functions. As in the above receptors, the membrane proximal region of the cytoplasmic domain is required for mitogenesis. In contrast, the membrane distal region, which is not required for mitogenesis, is required for the induction of genes that mediate acute phase responses (25) and for differentiating specific functions (26). G-CSF induces tyrosine phosphorylation of JAK2 and activation of its *in vitro* kinase activity.

The cytokines IL-6, CNTF, LIF, and OSM utilize receptors that share a gp130 or a related subunit termed the LIFRβ (27). This family of cytokines induces the tyrosine phosphorylation and activation of both JAK1 and JAK2 and, to a much more limited extent, tyk2 (28). Both JAK1 and JAK2 constitutively associate with gp130 or LIFRβ and this interaction requires the membrane proximal region of the cytoplasmic domain of the receptor. Interestingly, a particular cytokine could induce a distinct pattern of JAK1/JAK2/tyk2 phosphorylation in different cell lines. Thus, mechanisms may exist to modulate the interactions between gp130 or LIFRβ and specific kinases, thus allowing phenotypic differences in the responses of various cells to a specific cytokine.

Perhaps the most intriguing data relating the role of JAK family members with cytokine receptors concerns the IFN-α/β and IFN-γ receptors. These studies have utilized a series of mutants that were selected for their inability to respond to IFN-α, IFN-γ, or both. The U1 mutant fails to respond to IFN-α while retaining the ability to respond to IFN-γ. This mutant is defective in tyk2 and tyk2 can restore the ability to respond to IFN-α (18). Another mutant, termed γ-1, is defective in IFN-γ signaling but retains a normal response to IFN-α. Recently it has been found that the ability of this mutant to respond to IFN-γ could be restored by introducing JAK2 (29), thus implicating JAK2 in the IFN-γ pathway. In this case it was also demonstrated that IFN-γ induces the tyrosine phosphorylation of JAK2 and activates its *in vitro* kinase activity comparable to other cytokines.

The above data demonstrated that tyk2 and JAK2 were essential for IFN-α and IFN-γ signaling, respectively. However, another mutant, U4, also implicated JAK1. The U4 mutant lacked the ability to respond to either IFN-α or IFN-γ. The responsiveness to both IFN-α and IFN-γ could be restored by introducing JAK1 (30), thus demonstrating that both JAK1 and tyk2 are essential for an IFN-α response, while both JAK1 and JAK2 are essential for an IFN-γ response.

The requirement for two kinases initially suggested that there may exist a kinase cascade. However, this does not appear to be the case. Neither IFN-γ stimulation of the γ-1 mutant or IFN-α stimulation of the U1 mutant causes the tyrosine phosphorylation or activation of JAK1; thus, JAK1 is not "upstream" of either JAK2 or tyk2. Conversely, IFN-α or IFN-γ stimulation of the U4, the JAK1 deficient mutant, do not cause phosphorylation or activation of tyk2 or JAK2, respectively. Reconstitution in any of the mutants restores the ability to phosphorylate and activate both kinases. The current hypothesis to explain these results is that two kinases must exist in the receptor complex. This may occur through the ability of receptor subunits to bind to specific JAK family members.

The above data provide a compelling argument to suggest that, in general, the receptors of the cytokine receptor superfamily utilize one or more members of the JAK family of cytoplasmic kinases. However, there are notable exceptions. In particular, extensive efforts to find tyrosine phosphorylation or activation of JAK1, JAK2, or tyk2 in the response of T cells to IL-2 or IL-4 have failed (Quelle F, Witthuhn B, Ihle JN, unpublished data). Thus, either these cytokines do not utilize JAK family kinases or there exists as of yet unidentified members of the JAK family that these cytokines specifically require for signaling. Given the extensive similarity of the IL-2/IL-4 receptors and responses, it is most likely that another JAK family member exists. Indeed, by PCR amplification approaches, potentially new members of the JAK family have been identified and efforts are in progress to assess their role in IL-2/IL-4 signaling.

JAK Substrates Include a Family of Proteins That Regulate Gene Transcription

The general involvement of JAK family members in signaling has additional implications. Extensive studies have been done on the mechanisms by which interferons regulate gene expression (31–37). In particular, gene activation in response to IFN-α/β involves the participation of three gene products in a transcriptional activation complex termed ISGF3. The

48-kDa, ISGF3-γ component binds DNA, while the 113- and 91/84-kDa proteins of the ISGF3-α component are required for transcriptional activation. Following IFN-α binding, the 113- and 91/84-kDa proteins are rapidly tyrosine phosphorylated and acquire the ability to form a complex with the 48-kDa component which migrates to the nucleus and activates transcription of genes containing an interferon stimulated response element (ISRE).

The cellular response to IFN-γ also involves the phosphorylation of the p91-kDa substrate but not of the 113-kDa protein. Following phosphorylation, the 91-kDa protein migrates to the nucleus and participates in DNA-binding complexes that recognize gamma activated sequences (GAS) in genes that are transcriptionally regulated by IFN-γ. These complexes do not contain the 48-kDa, ISGF3-γ subunit but may contain other proteins involved in DNA binding.

There are two important, emerging concepts that build upon the above data. First, a number of recent studies have begun to suggest that the 91-kDa, ISGF3-α subunit is more generally involved in cell signaling. In particular, the 91-kDa protein is phosphorylated in the response to epidermal growth factor (38–41) and mediates activation of the c-*fos* gene through interaction with a GAS related site termed the serum inducible element (SIE). Secondly, it is evident that cytokines (42), as well as EGF (39) can induce novel factors that are tyrosine phosphorylated and bind GAS sequences. This has led to the hypothesis that there may be a family of proteins related to the 91- and 113-kDa proteins that participate in cytokine regulation of gene expression. Indeed, we have cloned the gene for a protein that is 52% identical to the 91-kDa, ISGF3-α subunit which is uniquely expressed in myeloid cells (Yamamoto K, Ihle JN, in preparation).

In summary, the available data suggest a general model for cytokine regulation of gene expression involving receptors of the cytokine receptor superfamily, JAK family members, and members of a family of proteins that share in common the ability to be activated by tyrosine phosphorylation and participate in complexes to activate gene transcription. Future studies will define more precisely the sequences in the membrane proximal, box1/box2, regions of the receptor cytoplasmic domains that are responsible for the association of the receptor and particular JAK family members. Similarly, it will be of considerable interest to define the regions of the JAK family members that bind to the receptors. These interactions are clearly essential for cytokine responses and are potential targets for rational drug development. Equally of interest will be the characterization of additional members of the JAK family and assessing their role in cytokine responses, particularly the responses to IL-2/IL-4. Lastly, it is very likely that a large family of 91/113-kDa related proteins exist which are substrates of one, or more, JAK family members, and which may regulate genes containing not only ISRE or GAS elements but perhaps others as well.

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