Kinases of the Jak family (Jak1/2/3 and Tyk2) interact with the membrane proximal domain of different cytokine receptors and play a critical role in the activation of cytokine and growth factor signaling pathways. In this report we demonstrate that both the Box 1 and Box 2 motif collaborate in the association and activation of Jak1 by type I interferons. Mutational analysis of the β chain of type I interferon receptor (IFNαRβ1/IFNAR2) revealed that Box 1 plays a more significant role in activation than in the association with Jak1. On the contrary, the Box 2 motif contributes more to the association with Jak1 than to kinase activation. Additionally, the study of the Jak1 binding sites on the IL2 receptor β (IL2Rβ), IFNγRα/IFNGR1, and IL10Rα/IL10R1 chains suggests that cytokine receptors have two different kinds of interaction with Jak1. One form of interaction involves the Box 1 and the previously described Box 2 motif, which we now designate as Box 2A, characterized by the VEV1 and LEVL sequences present in IFNαRβ1/IFNAR2 and IL2Rβ subunits, respectively. The second form of interaction requires a motif termed Box 2B, which is present in the IFNγRα/IFNGR1 (SILLPKS) and IL10Rα/IL10R1 (SVLILFKR) chains. Interestingly, Box 2B localizes close to the membrane region (8–10 amino acids from the membrane) similar to Box 1, whereas Box 2A is more distal (38–58 amino acids from the membrane).

Activation of kinases of the Jak family (Jak1, Jak2, Jak3, and Tyk2) plays a pivotal role in signaling through cytokine receptors, including interferons (IFN) (1–3). The membrane proximal domain of cytokine and IFN receptors constitutively interacts with Jak kinases (1–3). The interaction between a given receptor and Jak is not specific, because the same Jak can associate with and be activated by different receptors. However, the absence of a distinct Jak cannot be compensated by another Jak as has been demonstrated by the cytokine-specific phenotypes observed in animals with targeted disruptions of these kinases (4–8).

The interaction between Jak2 and single subunit cytokine receptors (i.e. erythropoietin receptor (EPOR), growth hormone receptor, and prolactin receptor (PRL-R)) as well as heterodimeric receptors (IFNγR and granulocyte-macrophage colony stimulating factor receptor/interleukin-3 and -5 receptors) has been extensively characterized (9, 10). The membrane proximal region of cytokine receptors that interact with Jak2 contains a highly conserved motif termed Box 1 (see Fig. 1E), which is formed by a proline-rich sequence located 6–10 amino acids after the transmembrane domain (11–13). The consensus sequence for the Box 1 motif present in single-subunit cytokine receptors can be described as follows: φφDXφL/VPXE/KE (φ = hydrophobic residues). It is also important to point out that a region within the cytoplasmic domain C-terminal from Box 1 contains a second motif, Box 2, which is not so well defined. The amino acid sequence more frequently found in the Box 2 motif is (VL)E/EVL/L represented by φφDXφL (see also Fig. 1E). The Box 2 motif is also present in single-chain cytokine receptors and is required in some cases for full activation of Jak2 (14).

Interestingly, the binding sites for Jak1, Jak3, and Tyk2 are not so well defined. The Tyk2 binding site on the α chain of IFNαR (or IFNAR1) has only distant homology with other cytokine receptors (15). Binding studies with IFNγR and IFNγ and several cytokines, including the IL6 (IL6, leukemia inhibitory factor, ciliary neurotrophic factor, oncostatin M) and IL2 families (IL2, IL4, IL7, IL9, and IL15) (17–25). The Jak1 binding site has been studied for a few cytokine receptors and appears to be rather heterogeneous. For example, the sequence 266LPKS269 of the α chain of IFNγR (or IFNGR1), which has at best distant similarity to Box 1, revealed that only Pro267 was important for Jak1 binding (19). In the case of the IL2Rβ chain, a sequence with some similarity to Box 1 and Box 2 appear to be important for Jak1 binding (26). Mutations of the Box 2 motif in gp130 and IL2Rβ chains affect not only binding but also activity (26–28). In comparison, initial studies of the Box 1 of the IFNαRβL (or IFNAR2) chain suggested that this motif by itself has a minimum contribution to Jak1 binding (21). Interestingly, the Box 1 and Box 2 motifs of IFNαRβL are separated by only 7 amino acids, whereas the distance between these two motifs in the IL2Rβ and gp130 is 38 and 31 residues, respectively. Although some of the cytokine receptor subunits that activate Jak1 have Box 1 and/or Box 2 motifs (i.e. gp130, IL2Rβ, and IFNαRβL), others such as the IL10Rα, and possibly...
the IFNγRα, do not have easily definable Box 1 or Box 2 motifs. This variability in the Jak1 binding sites of cytokine receptors also appears to be accompanied by differences on the corresponding receptor binding surfaces of Jak1 (29). Although all cytokine receptors require the JH7 and part of the JH6 domains of Jak1 for activation, distinct cytokine receptors interact with a second region of Jak1 that is receptor-specific. For example, the second area of interaction for the IL10Rα is JH3, whereas IL2Rβ and IL4Rα interact with JH4 and JH5–6, respectively (29).

This variability in the Jak1 binding site among cytokine receptors suggests that Jak1 could interact with different motifs in distinct cytokine receptors. This type of model would be important to develop therapeutic agents that may block Jak1 binding and/or function within the context of some cytokine systems without disturbing Jak1 activation by others. In this report, we sought to address whether there are differences in Jak1 binding sites among cytokine receptors. We characterized the kinase binding domain for two different types of receptors that activate Jak1: (a) receptors such as the IFNαRβL and IL2Rβ that contain Box 1 and Box 2 motifs and (b) receptors that do not have either Box 1 or Box 2 motifs (i.e. IFNγRα and IL10α). Although in the case of the IFNαRβL Box 2 appears to contribute to the majority of Jak1 binding, its mutation did not affect Jak1 activation by IFNα. Surprisingly, some mutations of Box 1 such as Pro289 and Pro291 affected Jak1 binding and activation, whereas other alterations such as the addition of a Pro in position 292 (N292P) or the inversion of Box 1 of IFNαRβL did not affect Jak1 binding but impaired Jak1 activation. The Box 2 motif of the IL2Rβ was also critical for binding to Jak1, whereas Box 1 cannot interact with Jak1 in the absence of Box 2.

Interestingly, IL10Rα and IFNγRα, which do not contain either Box 1 or Box 2 motifs, associate with Jak1 through a different motif that is localized closer to the transmembrane region. Thus, these data suggest that Jak1 interacts with cytokine receptors in two different manners. One form of interaction involves both Box 1 and Box 2 motifs (i.e. IL2Rβ and IFNαRβL chains). The Box 2A motif, previously described as Box 2, is represented by a sequence formed by hydrophobic, charged, hydrophobic, and hydrophobic amino acids (i.e. VEVI or LEVI). The second form of interaction, found in the IL10Rα and IFNγRα/IFNGR1 chains, involves Box 2B, a motif that is different from Box1 and Box 2A.

MATERIALS AND METHODS

IFNs and Antibodies—Human recombinant IFNα2 and IFNγ were kindly provided by Drs. M. Brunda (Hoffman-La Roche) and Ronald Borden (Scherer-Plough). The anti-phosphotyrosine antibody (4G10) and the anti-Jak2 serum were obtained from Upstate Biotechnology Inc. (Lake Placid, NY). The monoclonal antibodies against Jak1 and STAT1 were purchased from Transduction Laboratories (Lexington, KY).

GST Fusion Proteins—The following GST fusion proteins encoding full-length or truncated forms of the cytoplasmic domains of cytokine receptor subunits were used for this study (see Fig. 1). 1) GST-IFNαRβL265–515, GST-IFNαRβL265–375, and GST-IFNαRβL300–375, encoding full-length and the previously described Jak1 binding site with and without Box 1 of IFNαRβL (Fig. 1A, respectively). 2) GST-IL2Rβ encodes the full-length cytoplasmic domain of the IL2Rβ chain, whereas GST-IL2Rβ322 and GST-IL2Rβ265 encode truncations at amino acids 322 and 265 that containing Box 1 and Box 2, and Box 1 alone, respectively (Fig. 1B). 3) GST-IFNγRα and GST-IFNγRα (IFNγRα, short) encode the entire cytoplasmic domain of the IFNγRα/IFNGR1 chain and an N-terminal truncation that starts at amino acid 270 after the LPRS sequence important for Jak1 binding (Fig. 1C). 4) GST-IL10Rα and GST-IL10Rα299 correspond to the entire cytoplasmic domain of the IL10Rα chain and a form truncated at amino acid 299, respectively (Fig. 1D). Mutations introduced into the Box 1 motif of IFNαRβL chain (Fig. 1A) include Pro to Ala in positions 289, 291, and 294 (AWPFPNLEP), mutated residues are underlined. We also produced mutations that make Box 1 of IFNαRβL (AWPFPNLEP), closer to that observed in receptors that bind Jak2 such as the introduction of a Pro at position 292 (N292P, AWPFPNLEP), inversion of Box 1 motif from PXXPXP to PXXPXP (RV mutation, from AWFPFPNFLNP to AWPFPNFLNP and partial substitution of Box 1 of IFNαRβL for the Epor (AWPFPNFLNP to AWPFPNFLNP). Points mutations were generated by PCR using the overlap extension method or the QuikChange kit (Stratagene) and were confirmed by sequencing. All GST fusion proteins were produced as described previously (21), and the amount used in each experiment was determined by Coomassie Blue staining and/or immunoblotting with an anti-GST monoclonal antibody (Transduction Laboratories).
Jak1 Binding Domains in Cytokine Receptors

300–375, but not in the context of the full-length receptor, suggests that one or more regions within IFNαRβL other than amino acids 300–375 should also contribute to Jak1 binding and activation.

Mutations of the Box 1 Motif of IFNαRβL Affect Jak1 Activation—The logical candidate to contribute or collaborate with Box 2 in Jak1 binding and activation is the Box 1 motif. The minimal Jak1 binding previously identified with the Box 1 motif (21), in the absence of Box 2, could be sufficient to support Jak1 binding and activation in the context of a full-length receptor containing Box 2 mutations. Therefore, we next assessed the contribution of the Box 1 motif to Jak1 binding by introducing mutations within a GSTβL265–375 that also contains the Box 2 motif. Fig. 3 shows that mutation of Pro290 (lane 3) and Pro291 (lane 4) to Ala in Box 1 eliminated or decreased the association of IFNαRβL with Jak1, respectively (compare lane 2 with lanes 3 and 4), whereas the mutation Pro294 to Ala had no effect (compare lanes 2 and 6). Mutations Asn292 to Pro, EPOR, or RV, which we used in an attempt to make Box 1 similar to the one that binds Jak2 (PXXPXP), did not have an effect on Jak1 binding (lanes 5, 7, and 8, respectively). The effect on Jak1 binding observed by introducing mutations within Box 1 confirm that this motif plays a role in the interaction with Jak1 (21).

To determine the role of Box 1 on Jak1 activation, mutations of Box 1 were introduced into full-length IFNαRβL and ex-
pressed using a retroviral system. Clones were selected using G-418, and expression of the different constructs was confirmed by fluorescence-activated cell sorting analysis using a retroviral system. Clones were selected using G418 (500 μg/ml), expression of the mutant receptor in different clones was assessed using a monoclonal antibody directed against the β chain of IFNαR (IFNaRβ1, dashed line) or control IgG2a (solid line) as previously described (32).

Fig. 4. Expression of IFNaRβ1 Box 1 mutants in mouse L-929 cells. Retroviral supernatants were packaged in BOSC23 or PA317 cells and used to infect LpR cells as described under “Materials and Methods.” After selection in G418 (500 μg/ml), expression of the mutant receptor in different clones was assessed using a monoclonal antibody directed against the β chain of IFNαR (IFNaRβ1, dashed line) or control IgG2a (solid line) as previously described (32).

Fluorescence Intensity

Fig. 4. Expression of IFNaRβ1 Box 1 mutants in mouse L-929 cells. Retroviral supernatants were packaged in BOSC23 or PA317 cells and used to infect LpR cells as described under “Materials and Methods.” After selection in G418 (500 μg/ml), expression of the mutant receptor in different clones was assessed using a monoclonal antibody directed against the β chain of IFNαR (IFNaRβ1, dashed line) or control IgG2a (solid line) as previously described (32).

Fig. 5. Mutations of Box 1 of IFNoRβL affect Jak1 activation. A, cell lines expressing different mutations of Box 1 were treated with huIFNα2 (ha) for 15 min at 37 °C. Cell lysates were immunoprecipitated (IP) and Western blotted (WB) with the indicated antibodies. MulIFN4 (ma) was used as a positive control, because it activates the endogenous mouse receptor. B, mutation N292P of IFNoRβL does not affect Jak1 binding. Cell lysates obtained from L-929 stable transfectants expressing mutations N292P and EPOR were immunoprecipitated with polyclonal antibodies against IFNoRβL (βL), Jak1 (J1), or normal rabbit serum (NR) and immunoblotted with an anti-Jak1 monoclonal antibody.

Fig. 5. Mutations of Box 1 of IFNoRβL affect Jak1 activation. A, cell lines expressing different mutations of Box 1 were treated with huIFNα2 (ha) for 15 min at 37 °C. Cell lysates were immunoprecipitated (IP) and Western blotted (WB) with the indicated antibodies. MulIFN4 (ma) was used as a positive control, because it activates the endogenous mouse receptor. B, mutation N292P of IFNoRβL does not affect Jak1 binding. Cell lysates obtained from L-929 stable transfectants expressing mutations N292P and EPOR were immunoprecipitated with polyclonal antibodies against IFNoRβL (βL), Jak1 (J1), or normal rabbit serum (NR) and immunoblotted with an anti-Jak1 monoclonal antibody.

Identification of Jak1 Binding Sites on the IL2Rβ—Zhu et al. (26) previously reported that some residues within Box 1 and the first Leu within Box 2 affected Jak1 binding. We next determined whether Box 1 and Box 2 of the IL2Rβ play the same role in the association with Jak1. Because Box 1 of IFNoRβL alone is unable to bind substantial amounts of Jak1, we truncated a GSTIL2Rβ after the Box 1 motif to determine if Box 1 of IL2Rβ alone was able to associate with Jak1. Fig. 6 shows that Jak1 interacts with the full-length cytoplasmic domain of IL2Rβ (lane 2) and with a fusion protein truncated at residue 322 (lanes 3 and 4), which contains the Box 1 and Box 2 motifs. However, Jak1 does not interact with a more proximal truncation (amino acid 265) that only includes the Box 1 motif (lane 5). This result indicates that Box 1 of the IL2Rβ, like Box 1 of IFNoRβL, is not sufficient for Jak1 binding.

Because the Box 2 motifs of IL2Rβ and IFNoRβL are very similar (LEVL and VEV1, respectively), we also wanted to
assess the contribution of the different amino acids of Box 2 of IL2Rβ to Jak1 binding. Fig. 6B (lane 2) shows that as in the case of IFNαRβL, mutation of the first hydrophobic residue of this motif (Leu299) results in a marked decrease in Jak1 binding as previously reported (26), whereas mutations of the two other hydrophobic amino acids (Val301, Glu300) have a less significant effect (lanes 3–5) on the interaction with the kinase. The lower panel (Fig. 6B, WB: GST) shows that similar amounts of GST fusion proteins were used. These data suggest that the function of the Box 1 and Box 2 motifs of IL2Rβ and IFNαRβL is very similar. Box 1 by itself does not sustain efficient Jak1 binding, and equivalent amino acids within Box 2 are important for the association with Jak1. However, they differ in the impact that mutations of Box 2 have on Jak1 activation, because substitution of Leu299 of IL2Rβ completely abrogated Jak1 activation (26, 28), whereas the same mutation of IFNαRβL did not have any effect (see “Discussion”).

The IFNγRα and IL10Rα Chains Use a Different Jak1 Binding Motif—We finally sought to address which regions are important for Jak1 binding in receptors that do not contain Box 1 or Box 2 motifs. The previously reported Jak1 binding site of the IFNγRα is localized very close to the transmembrane region and does not resemble the Box 1 motif of receptors that activate Jak2 or even the less conserved Box 1 of IFNαRβL or IL2Rβ (19). We produced GST fusion proteins encoding the entire cytoplasmic domain and a deletion of the cytoplasmic region containing the previously described Jak1 binding site (19) of the IFNγRα to confirm that the Jak1 binding site was indeed unique. Deletion of the region containing the LPKS sequence (IFNγRαS) completely abrogated Jak1 binding (Fig. 7A, compare lanes 1 and 2), confirming that the binding site for this kinase resides within the first 15 amino acids of the cytoplasmic domain of IFNγRα. This result suggests that Jak1 does not interact in the same manner with all cytokine receptors. For instance, IFNγRα has a Jak1 binding site that is very close to the transmembrane region (~10 amino acids) and is different from the typical Box 1 responsible for Jak2 binding, the less conserved Box 1 observed in IL2Rβ and IFNαRβL (Fig. 1E), and the Box 2 motif.

We finally determined the Jak1 binding sequence used by the IL10Rα chain, which, like the IFNγRα, contains neither a Box 1 nor Box 2 motif. Fig. 7B shows that GST fusion proteins, encoding the full-length IL10Rα, associate with Jak1 (Fig. 7, A and B, lanes 3 and 2, respectively). A fusion protein containing only the first 39 amino acids of the cytoplasmic domain of IL10Rα bound Jak1 as efficiently as the full-length receptor (Fig. 7B, lanes 3 and 4). These data suggest that, in the case of IFNγRα, the Jak1 binding site in the IL10Rα may be close to the transmembrane region.

To further define the residues involved in Jak1 binding, we performed mutagenesis using constructs in which 3 residues were simultaneously mutated to Ala. Two of these GST fusion proteins carrying mutation of residues 269–271 and 272–274 showed a significant decrease in Jak1 binding (Fig. 7C, lanes 3 and 4). A decrease in Jak1 binding observed with a construct containing mutations of amino acids 266–268 (lane 2) is due to lower amounts of this GST used in this experiment (Fig. 4C, lower panel). The sequence SVLFLKK in the 269–274 region of the IL10Rα is similar to SIILPKS (amino acids 263–269) found in the IFNγRα. These motifs are 8 and 10 amino acids from the membrane, respectively. In the previous characterization of the Jak1 binding site of IFNγRα (19), the first two hydrophobic residues (Ile281 and Ile282) were not included in the mutational analysis. We performed single point mutations of the first two hydrophobic amino acids present in this motif (280V/L270) and Phe272 in the IL10Rα. Phe272 is in the same position as Pro267 in the IFNγRα, which is critical for Jak1 binding. Fig. 7D shows that mutations of Leu270 and Phe272 (lanes 3–5), but not substitution of Val269 (lane 2), in IL10Rα significantly diminished

FIG. 6. The Box 2 motif of the IL2Rβ chain is important for Jak1 binding. A, GSTIL2Rβ wild type (lane 2) with truncation at amino acids 322 (lanes 3 and 4), and 265 containing only the Box 1 motif (lane 5) were used to pull-down Jak1 from U-266 lysates (upper panel). Jak1 was detected by immunoblotting with an anti-Jak1 monoclonal antibody. Pull down with GST alone and immunoprecipitation with an anti-Jak1 antibody were used as negative and positive control, respectively. Lower panel, Coomassie Blue staining of the different IL2Rβ chain proteins used in the upper panel. The migration of the different GST fusion protein is indicated (asterisk). B, similar experiment as in A, but using GSTIL2Rβ encoding single-amino acid substitutions of the Box 2 motif (upper panel). Lower panels, the filter was stripped and reprobed with an anti-GST monoclonal antibody to show that similar levels of GST fusion proteins were used.

FIG. 7. Characterization of the Jak1 binding site of IFNγRα and IL10Rα. A, Jak1 interact with the membrane proximal domain of IFNγRα. The GST–IFNγRαS (IFNγRαS, lane 2) encodes a deletion of the first 17 amino acids of the cytoplasmic domain containing the LPKS motif. U-266 cell lysates were used as the source for Jak1 protein. B, GST fusion proteins, encoding the full-length cytoplasmic domain of IL10Rα (lane 2) or truncated at amino acid 299 (lanes 3 and 4), were used to pull down Jak1 from U-266 lysates as in previous experiments. C and D, characterization of the amino acids important for Jak1 binding in the IL10Rα using constructs containing mutations of three (C) or one (D) amino acid to alanine. The indicated mutations were introduced in a GST fusion protein encoding the full-length cytoplasmic region of the IL10Rα chain. Lower panels (A–D), filters were stripped and reprobed with an anti-GST monoclonal antibody. The arrows in B show degradation products of the full-length GSTIL10Rα.
Jak1 binding. These data suggest that IL10Ra and IFNγRa interact with Jak1 in a similar manner, which differs from the interaction of Jak1 with IFNαRβL or IL2Rβ.

**DISCUSSION**

Ligand-induced dimerization of receptor subunits brings Jak kinases into proximity allowing resting or low activity kinases to become fully activated. This probably occurs through transphosphorylation of tyrosines in the activation loop. Therefore, the position of the kinases on cytokine receptor chains may be critical to allow the proper conformation after receptor dimerization. The Box 1 motif is a proline-rich motif (33) in a hydrophobic context present in most cytokine receptor chains that interact with Jak2. This motif is invariably positioned 6–12 residues from the transmembrane domain (Fig. 1E). The Box 1 motif on single-subunit cytokine receptors (i.e. GH-R, EPO, and PRL-R) (Fig. 1) consists of two hydrophobic amino acids, proline #1, any amino acid, a valine or isoleucine, proline #2, any amino acid, proline #3, and a charged residue. In the case of multichain cytokine receptors that interact with Jak2 such as IL3Rα, IL5Rα, GMCSFR, and IFNγRβ/IFNGR2, proline #3 may not be present (see Fig. 1, granulocyte-macrophage colony stimulating factor receptor, IL3Rα, and IFNγRβ).

The interaction between Jak1 and cytokine receptor subunits does not appear to be so well defined. In most cases, the Box 1 motif is not as conserved (i.e. IL2Rβ and IL4Ra; see Fig. 1E, Box 1) or not present (i.e. IL10Ra). In the case of the IFNγRa chain, analysis of a region proximal to the membrane (−15 residues) revealed that only mutation of Pro269, within a motif with little homology to Box 1, affects Jak1 binding and activation. In contrast, the initial mapping of the Jak1 binding site on the IFNαRβL chain revealed that a region more distal from the membrane was more important for Jak1 binding than the Box 1 motif (21). These differences led us to question whether the interaction between cytokine receptors and Jak1 follows different rules than those for the association between single subunit receptors and Jak2.

**Interaction between Jak1 and IFNαRβL or IL2Rβ**—The Box 1 and Box2 motifs of the IFNαRβL chain, unlike in the IL2Rβ, are very close to each other and probably contribute to the formation of the binding site. It is likely that the Box 2 motif has the largest area of contact with Jak1, because GST fusion proteins that do not contain the Box 1 motif bind Jak1 almost as efficiently as wild type (21). However, the contribution of Box 1 to the formation of the Jak1 binding site is demonstrated by the finding that mutations of Pro269 or Pro291 decreases the interaction with this kinase even in the presence of Box 2. However, even though both motifs collaborate in Jak1 binding, Box 1 appears to be more important for the regulation of kinase activity. This is supported by the finding that kinase activation was affected even by mutations of Box 1 that did not affect Jak1 binding.

The Box 2 motifs in IL2Rβ and IFNαRβL are very similar, and Ala mutations of either motif appear to have equivalent effects on Jak1 binding. Moreover, the use of GST fusion proteins that contain only Box 1 revealed that neither Box 1 of IL2Rβ nor IFNαRβL could bind substantial amounts of Jak1 by itself. However, unlike IL2Rβ (26, 28), mutations of Box 2 of IFNαRβL did not affect Jak1 activation. It is possible that the distance separating the Box 1 and Box 2 motifs in these receptors (7 and 38 residues for IFNαRβL and IL2Rβ, respectively) results in a different type of interaction with Jak1. This type of model is supported by the finding that slightly different regions of Jak1 are required to associate with the IFNαRβL and IL2Rβ chains (29).

**IFNγRa and IL10Ra Use a Different Jak1 Binding Motif**—Unlike IFNαRβL and IL2Rβ, IFNγRa and IL10Ra do not have an identifiable Box 1 or Box 2 motif. This prompted us to further characterize the interaction between Jak1 and these receptors. Our results indicate that the IL10Ra has a Jak1 binding site close to the transmembrane region that has some degree of similarity with IFNγRa. Moreover, mutation of Phe272 of IL10Ra, which is the equivalent to Pro269 in IFNγRa, significantly decreased the interaction with Jak1. Altogether, these data suggest that there are at least two different types of interactions between Jak1 and cytokine receptors. First, cytokine receptor such as IL2Rβ and IFNαRβL interact with Jak1 through the Box 1 and Box 2 motifs that we will call Box 2A characterized by a hydrophobic-charged-hydrophobic-hydrophobic sequence. The Box 1 in these receptors is slightly different from Box 1 present in single subunit receptors that activateJak2 (see Fig. 1E). Second, other cytokine receptors such as IL10Ra and IFNγRa use a different motif that is close to the membrane that we will call Box 2B. The Box 2B motif is characterized by a core of 4 hydrophobic resiudles flanked by a Ser and charged residues. However, mapping of the regions of Jak1 involved in the interaction with cytokine receptors strongly suggest that a region different from the Box 2B motif of the IL10Ra also interacts with Jak1 (29). In the case of IFNγRa, a GST fusion protein, encoding the entire cytoplasmic domain except Box 2B, failed to interact with Jak1. These data suggest that Box 2B, at least in IFNγRa, should be sufficient to sustain Jak1 binding and probably activation.

Contrary to what was observed with the interaction between Jak2 and Box 1, the position of the Jak1 binding domain within the cytoplasmic region of cytokine receptors was more variable. This raises the hypothesis that the positioning of the kinase within the cytoplasmic domain may be important for correct kinase alignment after receptor dimerization, which would allow transphosphorylation of the tyrosines in the activation loop. In single subunit receptors, ligand binding results in homodimerization and, therefore, Jak2 is always at the same position in both members of the dimer. However, in heterodimeric receptors, positioning may be more critical. In this scenario, the Jak1 binding site in IFNγRa is closer to the membrane to produce a correct alignment with Jak2, which also interacts with a binding site on the IFNγRβL chain that is close to the membrane. This suggests that the proximal Jak1 binding site on IFNγRa evolved to pair the Jak2 site present in IFNγRa. The Tyk2 (34) and Jak1 binding sites on the α and βL chains of IFNαR are also at similar distances from the membrane (32 and 38 amino acids, respectively). This model would predict that the Tyk2 binding site on the IL10Ra (CRF4) (35, 36) should be proximal to the membrane to match the membrane proximal Jak1 site present on the IL10Ra chain. It should be considered that what we refer as “distance from the membrane” is only a relative measurement, because we do not know the secondary and tertiary structure of the cytoplasmic domain of these receptors. The finding that dimerization of the EPOR, for example, is not enough for activation may support the role of kinase orientation or stereo conformation in receptor activation (37).

**REFERENCES**

1. Schindler, S., and Darnell, J. J. E. (1995) *Annu. Rev. Biochem.*, 64, 621–651
2. Ihe, J. N. (1995) *Nature* 377, 591–594
3. Ihe, J. E. (1996) *Cell* 84, 331–334
4. Bodig, S. J., Mora, M. A., White, J. M., Lampe, P. A., Riley, J. K., Arthur, C. D., King, K. L., Sheehan, K. C. F., Yin, L., Pennica, D., Johnson, J. E. M., and Schreiber, R. D. (1998) *Cell* 93, 373–383
5. Farganas, E., Wang, D., Steropoditis, D., Topham, D. J., Marine, J.-C., Te-glund, S., Vanin, E. F., Bodner, S., Colomoni, O. R., van Deursen, J. M., Grosfeld, G., and Ihe, J. E. (1998) *Cell* 93, 385–395
6. Neubauer, H., Cumanu, A., Muller, M., Wu, H., Huffstadt, U., and Pfeffer, K. (1998) *Cell* 93, 397–409
7. Shimoda, K., Kato, K., Aoki, K., Matsuda, T., Miyamoto, A., Shihabumi, M., Yamashita, M., Numata, A., Takase, K., Kobayashi, S., Shibata, S., Asano,
Y. Gondo, H. Sekiguchi, K. Nakayama, K., Nakayama, T., Okamura, T., Okamura, S., Niyo, Y., and Nakayama, K.-i. (2000) Immunity 13, 561–571
8. Karaghiosoff, M., Neubauer, H., Lasnigc, C., Kovarik, P., Schindler, H., Firchner, H., McCoy, B., Bogdan, C., Decker, C., Brem, G., Pfeffer, K., and Muller, M. (2000) Immunity 13, 549–560
9. Witthuhn, B. A., Quelle, F. W., Silvennoinen, O., Yi, T., Tang, B., Miura, O., and Ihle, J. N. (1993) Cell 74, 227–236
10. He, T.-C., Jiang, N., Zhou, H., Quelle, D. E., and Wojchowski, D. M. (1994) J. Biol. Chem. 269, 18291–18294
11. Miura, O., Cleveland, J. L., and Ihle, J. N. (1993) Mol. Cell. Biol. 13, 1788–1795
12. Hackett, R. H., Wang, Y.-D., Sweitzer, S., Feldman, G., Wood, W. I., and Coloni, O. R. (1993) J. Biol. Chem. 268, 12617–12623
13. Joneja, B., and Wojchowski, D. M. (1997) J. Biol. Chem. 272, 11176–11184
14. Coloni, O. R., and Domanski, P. (1997) J. Biol. Chem. 272, 11176–11184
15. Yan, H., Krishnan, K., Lim, J. T. E., Cortillo, L. G., and Kroewski, J. J. (1996) Mol. Cell. Biol. 16, 2074–2082
16. Coloni, O. R., Uyttendaele, H., Domanski, P., Yan, H., and Kroewski, J. J. (1994) J. Biol. Chem. 269, 3518–3522
17. Stahl, N., Boulton, T. G., Farrugiala, T. Ip, N. Y., Davis, S., Witthuhn, B. A., Quelle, F. W., Silvennoinen, O., Barbier, G., Pellegrini, S., Ihle, J. N., and Yanceopoulos, G. D. (1994) Science 263, 92–95
18. Lotticker, C., Wegenka, U. M., Yuan, J., Buschmann, J., Schindler, C., Ziemiecki, A., Harpur, A. J., Wilks, A. F., Yasukawa, K., Taga, T., Kishimoto, T., Barbier, G., Pellegrini, S., Sendtner, M., Heinrich, P. C., and Horn, F. (1994) Science 263, 89–92
19. Kaplan, D. H., Greenlund, A. C., Tanner, J. W., Shaw, A. S., and Schreiber, R. D. (1996) J. Biol. Chem. 271, 9–12
20. Sakatsume, M., Igurashi, K.-i., Winestock, K. D., Garotta, G., Larson, A. C., and Fishkind, D. S. (1996) J. Biol. Chem. 270, 17528–17534
21. Domanski, P., Nadeau, O. W., Fish, E., Kellum, M., Platianis, L. C., Pitha, P., and Coloni, O. R. (1997) J. Biol. Chem. 272, 26388–26393
22. Muller, M., Briscoe, J., Laxten, C., Guschin, D., Ziemiecki, A., Silvennoinen, O., Harpur, A. G., Barbier, G., Witthuhn, B. A., Schindler, C., Pellegrini, S., Wilks, A. F., Ihle, J. N., Stark, G. R., and Kerr, I. M. (1993) Nature 366, 129–135
23. Miyazaki, T., Kawahara, A., Fuji, H., Nakagawa, Y., Minami, Y., Liu, Z.-J., Oishi, I., Silvennoinen, O., Witthuhn, B. A., Ihle, J. N., and Taniguchi, T. (1994) Science 266, 1045–1047
24. Yin, T., Tsang, M. L.-K., and Yang, Y.-C. (1994) J. Biol. Chem. 269, 10944–10947
25. Nakamura, Y., Russell, S. M., Mess, S. A., Friedmann, M., Eros, M., Francois, C., Jacques, Y., Adelstein, S., and Leonard, W. J. (1994) Nature 363, 330–333
26. Zhu, M.-h., Berry, J. A., Russell, S. M., and Leonard, W. J. (1998) J. Biol. Chem. 273, 10719–10725
27. Narazaki, M., Witthuhn, B. A., Yoshida, K., Silvennoinen, O., Yasukawa, K., Ihle, J. N., Kishimoto, T., and Taga, T. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 2285–2289
28. Goldsmith, M. A., Xu, W., Amaral, M. C., Kuczuk, E. S., and Greene, W. C. (1994) J. Biol. Chem. 269, 14698–14704
29. Usacheva, A., Kotenko, S., Witte, M. M., and Coloni, O. R. (2002) J. Immunol. 169, 1302–1308
30. Coloni, O. R., and Domanski, P. (1993) J. Biol. Chem. 268, 10895–10899
31. Kotenko, S. V., Izotova, L. S., Mirochnitchenko, O. V., Lee, C., and Pestka, S. (1999) Proc. Natl. Acad. Sci. 96, 5007–5012
32. Usacheva, A., Smith, R., Baida, G. E., Croze, E., and Coloni, O. R. (2001) J. Biol. Chem. 276, 22948–22953
33. O’Neal, K. D., and Yu-Lee, L.-Y. (1993) Lymphokine Cytokine Res. 12, 309–312
34. Yan, H., Piazza, F., Krishnan, K., Pine, R., and Kroewski, J. J. (1998) J. Biol. Chem. 273, 4046–4051
35. Kotenko, S. V., Izotova, L. S., Pollack, B. P., Muthukumaran, G., Paukku, K., Silvennoinen, O., Ihle, J. N., and Pestka, S. (1996) J. Biol. Chem. 271, 17174–17182
36. Kotenko, S. V., Krause, C. D., Izotova, L. S., Pollack, B. P., Wu, W., and Pestka, S. (1997) EMBO J. 16, 5884–5903
37. Livnah, O., Johnson, D. L., Stura, T. F., Farrell, F. X., Barbone, F. P., You, Y., Liu, K. D., Goldsmith, M. A., He, W., Krause, C. D., Pestka, S., Jolliffe, E. K., and Wilson, I. A. (1998) Nat. Struct. Biol. 5, 993–1004