A Region of the β Subunit of the Interferon α Receptor Different from Box 1 Interacts with Jak1 and Is Sufficient to Activate the Jak-Stat Pathway and Induce an Antiviral State*

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Coexpression of the α and βL subunits of the human interferon α (IFNa) receptor is required for the induction of an antiviral state by human IFNa. To explore the role of the different domains of the βL subunit in IFNA signaling, we coexpressed wild-type α subunit and truncated forms of the βL chain in L-929 cells. Our results demonstrated that the first 82 amino acids (AAs) (AAs 265–346) of the cytoplasmic domain of the βL chain are sufficient to activate the Jak-Stat pathway and trigger an antiviral state after IFNα2 binding to the receptor. This region of the βL chain, required for Jak1 binding and activation, contains the Box 1 motif that is important for the interaction of some cytokine receptors with Jak kinases. However, using glutathione S-transferase fusion proteins containing amino- and carboxyl-terminal deletions of the βL cytoplasmic domain, we demonstrated that the main Jak1-binding region (corresponding to AAs 300–346 on the βL subunit) is distinct from the Box 1 domain (AAs 287–295).

Type I interferons (IFNs) bind to a multimeric receptor (type I IFN-R, IFNR, or IFNR0R) composed of at least two subunits designated as α (1–5), and β (6, 7). The IFNR0R cDNA (8) corresponds to the short form of the β subunit (βS), whereas cDNAs for the long form (βL) have been recently isolated by two different groups (9, 10). Transcripts and mature proteins for both βS and βL are found in most cell lines, although there is a large excess of βL protein when compared with βS.² Lutfalla et al. (10) have also shown that the short and long forms of the β subunit are generated by alternative splicing of the same gene. Interestingly, the type I IFN-binding proteins are not limited to the cellular components described above, because we and others have found a vaccinia virus-encoded protein capable of binding type I IFNs as well as blocking type I IFN signaling (11, 12).

Coexpression of the α subunit with either βL or βS in mouse L-929 cells reconstitutes the high affinity receptor, whereas either form of the β chain expressed independently binds type I IFNs with low affinity. Additional studies using these transfectants show that the IFNa response can be reconstituted in mouse cells only when the α chain is coexpressed with the βL subunit rather than βS, indicating that both the α and βL subunits are indispensable for IFNA signaling (9). Similarly, only the long form of the β subunit is able to complement the mutant cell line 5U5A that is defective in the IFNα pathway (10).

IFNs, cytokines, and growth factors activate the Jak-Stat pathway; more specifically, type I IFNs activate Jak1, Tyk2, Stat1, Stat2, and Stat3 (for recent reviews, see Refs. 13 and 14). Ligand binding to the cytokine receptor subunits activates tyrosine kinases of the Jak family that are constitutively associated with different cytokine receptors. It has been demonstrated that Jak2 binds to the Box 1 motif present in the membrane proximal region of the erythropoietin, prolactin, growth hormone, and granulocyte macrophage colony-stimulating factor receptors (15–21). In the case of the α subunit of the type I IFN-R, there is no conserved Box 1 motif, and Tyk2 interacts with a distinct region different from that of Box 1 (22). Furthermore, in those cytokine receptors that activate Jak1, the region that interacts with this kinase has not been clearly defined. The presence of a Box 1 motif in the membrane proximal region of the βL subunit of the type I IFN-R suggested a possible role for this motif in the binding and/or activation of Jak1 by this receptor subunit. We therefore sought to determine the role of the Box 1 and other domains of the βL chain in type I IFN signaling using two strategies: (a) we stably cotransfected mouse L-929 cells with the wild-type α subunit and various truncated forms of the βL chain to study in vitro signaling processes; and (b) we produced GST fusion proteins with deletions of the βL chain to demonstrate interactions in vitro. Our data indicate that a minimal region of the βL subunit cytoplasmic domain, specifically AAs 265–346, is required to activate Jak1 and induce an antiviral state. Using GST fusion proteins we show that this region contains a Jak1 binding domain (AAs 300–346) that is different from the Box 1 motif (AAs 287–295). The interaction between the βL chain and Jak1 is direct and does not require binding of IFNα2 to the receptor, although ligand binding slightly increases the amount of Jak1 associated with the βL chain. These results suggest that the homologous Jak kinases may interact with different motifs.
within the proximal region of the cytoplasmic domain of cytokine receptors.

EXPERIMENTAL PROCEDURES

IFNα, Antibodies, and Antiviral Assays—Human recombinant IFNα2 was kindly provided by Dr. M. Brunda (Hoffman-La Roche) and Dr. Paul Trotta (Schering-Plough, Union, NJ). The anti-phosphotyrosine antibody (4G10) was obtained from Upstate Biotechnology, Inc. (Lake Placid, NY). Monoclonal antibodies against Tyk2, Jak1, and Stat1 were purchased from Microvision Laboratories (Lexington, KY). The anti-α and anti-β subunits were kindly provided by Drs. D. Levy (New York University, New York, NY) and J. N. Ihle (St. Jude’s Children’s Hospital, Memphis, TN), respectively. Antiviral assays were performed as described previously (23).

Construction and Expression of Different Deletions of the βL Subunit of Type I IFN-R in Mouse L-929 Cells—These constructs were made by PCR using proofreading Vent polymerase and primers with an early termination codon at positions 300, 346, 360, or 417 (Fig. 1), respectively. The forward and reverse primers contained a BglII restriction site that was used for subcloning into the BamHI site of the pZipNeoSVX retroviral vector (constructs pZβL265–375, pZβL265–346, and pZβL265–306). The construct in which the βL chain was truncated at position 265 (pZβL265) was made by BamHI restriction endonuclease digestion of the 4A1 clone (9) followed by subcloning into the BamHI site of the pZipNeoSVX retroviral vector (Fig. 1). Sequencing of the pZβL265 construct revealed the addition of 13 amino acids (GYSMGHPA/PCKLP) before a termination codon in the pZipNeo vector. PCR mutations were confirmed by sequencing. Expression of the α and β subunits constructs in mouse L-929 cells was achieved by cotransfection of the ρZipNeoRα constructs and the pR4.cDNA constructs containing the α subunit (cell lines designated as LpRZ; i.e., LpZR265346, or LpZR265–306) or transfection of the βL subunit constructs into the LpRα cell line that corresponds to L-929 cells stably transfectcd with the pR4IFNαRα construct (cell lines designated as LpRZ, i.e., LpZβL265–346). Transfectants were selected in medium containing G418 (500 μg/ml) and hygromycin B (500 μg/ml), and individual clones were isolated and screened for receptor expression using affinity cross-linking and binding methods (see below).

Production of GST Fusion Proteins—The GSTαS55–655 encoding amino acids 465–557 of the α subunit of the type I IFN-R has been described previously (3). The constructs for the cytoplasmic domain of the βL and βαL forms of the β subunit were made by PCR amplification using a common forward primer (AAATGATGCCTGTTATATGC) for the common region of the cytoplasmic domain and a specific reverse primer for βL and the SP6 primer for βαL. The PCR products were digested with BamHI and subcloned into pGEX-KG vector to generate GSTβL, spanning the whole cytoplasmic domain of βL (amino acids 265–531) (8) and GSTβαL, corresponding to residues 265–462 of βαL (9). The GSTβL265–346 construct corresponding to the whole cytoplasmic domain of βL was generated by SalI/HindIII digestion of the PCR product and subcloned into the XhoI/HindIII restriction sites of pGEX-KG. The GSTβL265–462 construct was digested with SacI, SalI/SmaI, and NcoI to generate GSTβL265–375, GSTβL265–346, and GSTβL265–299, respectively (Fig. 1). GSTβL300–315 was produced by deletion of an NcoI restriction fragment (from the NcoI site in the pGEX-KG cloning site to the NcoI site at position 959 of the βL chain) from the GSTβL265–315 construct. The GST fusion proteins were produced in BL-21 cells (Novagen) and purified by affinity chromatography on glutathione-Sepharose (Pharmacia). 5–10 μg of the indicated GST fusion proteins were used for precipitations.

Immunoblotting—Cells were treated with different concentrations of the indicated IFNs for 15 min, rapidly centrifuged at 2,000 × g for 30 s in an Eppendorf microfuge, and subsequently lysed by buffers containing (20 mM Tris, pH 7.5, 50 mM NaCl, 10 mM sodium pyrophosphate, 20 mM NaF, 1 mM EDTA, 1 mM MgCl2, 1 mM diethiothreitol, 0.5% Triton X-100, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 100 mM phenylmethylsulfonyl fluoride, and 200 μM sodium orthovanadate). Immunoprecipitation and immunoblotting were performed as described previously (3).

Production of Recombinant Jak1 Proteins Using In Vitro Translation Systems—Jak1 cDNA was subcloned into the pGEM vector under the control of the T7 promoter, and [35S]methionine-labeled protein was produced using rabbit reticulocyte (Novagen) or wheat germ (Promega) in vitro translation kits.

Radioiodination of Type I IFNs, Competitive Displacements, and Affinity Cross-Linking—Radioiodination of IFNα2, competitive displacement assays, and affinity cross-linking procedures were performed as described previously (2).

Electrophoretic Mobility Shift Assay (EMSA)—Whole cell extracts were prepared as described previously (24) and analyzed by EMSA using end-labeled IFN-stimulated response element and m67SIE oligonucleotides to detect ISGF3 and c-sis-inducible factor complexes (24).

RESULTS

Expression and Characterization of L-929 Transfectants Expressing Truncation of the βL Subunit—To determine the biological relevance of disrupting the interactions of the βL subunit with the IFNα signaling machinery, we made constructs in which the βL chain was truncated at amino acids 462, 417, 360, or 300, respectively (Fig. 1). These βL chain constructs were coexpressed with the wild-type α subunit in mouse L-929 cells, and stable transfectants were selected with G418 and hygromycin B. We characterized the cell surface expression of the different human type I IFN-R chains by cross-linking 125I-labeled IFNα2 to the receptor, followed by immunoprecipitation with specific antibodies against the α, βL, or βαL subunits. Fig. 2 shows that the anti-α subunit antibody detects the α subunit and a high molecular weight complex (lanes 1, 4, 7, 10, 13, and 16) that includes the α and βL chains (9, 25). The anti-βL subunit antibody immunoprecipitates the βL chain (Fig. 2, 2, lanes 3, 6, 9, 12, 15, and 18) and the high molecular weight complex containing the α and βL chains (arrow) and also coprecipitates the α subunit as described previously (25). The signal corresponding to the α subunit is similar in most transfectants, suggesting that relatively equivalent amounts of high affinity receptors are expressed in the different transfectants. The differences in the intensity of the bands observed in immunoprecipitates performed with the anti-βL serum are likely due to the low efficiency of cross-linking of IFNα2 to this subunit (9) and to the fact that different epitopes recognized by this serum may not be equally exposed in the different mutants. As expected, a specific rabbit serum raised against the carboxyl-terminal region of the βL chain failed to react with any of the complexes (lanes 2, 5, 8, 14, and 17). In human U-266 cells (positive control), βL cross-linked to IFNα2 was observed in long exposures of the autoradiograms after immunoprecipitation with the anti-βL sera, confirming that the βL chain is expressed at much lower levels than βL in this cell line (comparing lanes 11 and 12). The anti-βL serum detects all truncated forms of the βL chain, except the most proximal at amino acid 300. However, the expression of this truncated form of βL 300 can be detected when cell lysates obtained after affinity cross-linking are directly resolved in an 8% SDS-PAGE (Fig. 2B).

Binding of radioiodinated IFNα2 to all transfectant cell lines was specifically blocked by the IFNαR1 monoclonal antibody.
that recognizes the extracellular domain shared by both forms of the \( \beta \) subunit (Fig. 2B, lane 1 and data not shown). These results indicate that these truncated forms of the \( \beta_L \) chain are expressed correctly on the cell surface.

No significant difference in the \( K_d \) for high affinity receptors was observed among the various cell lines (Table I). The \( K_d \) for high and low affinity receptors ranged from 22–91 pm and 0.5–5 nm, respectively. Most cell lines expressed between 2,400 and 4,600 high affinity sites/cell, except for LpRZ\( \alpha \) and LpRZ\( \beta \), which expressed slightly lower numbers (780 sites/cell). However, it has been previously demonstrated that stable transfectants expressing low numbers of high affinity receptors display a complete antiviral effect in response to human IFN\( \alpha \) and human IFN\( \beta \) (9).

**The First 82 Amino Acids of the Cytoplasmic Domain Are Sufficient to Elicit an Antiviral Response**—We first tested the ability of human IFN\( \alpha 2 \) to induce an antiviral state in the different transfectants. As can be seen in Table II, all of the transfectants were able to mount an antiviral response to human IFN\( \alpha 2 \), except for L-929 cells expressing human \( \beta_L \) chain truncated at amino acid 300. The antiviral effect induced by human IFN\( \alpha \)2 in transfectants expressing \( \beta_L \) chain truncated at residues 462, 417, 360, or 346 was comparable to that in incomplete antiviral effect in response to human IFN\( \alpha \). Most cell lines expressed low numbers of high affinity receptors display a complete antiviral effect induced by human IFN\( \alpha \)2 and human IFN\( \beta \).

**Activation of the Jak-Stat Pathway in L-929 Transfectants Expressing Truncated Forms of the Human \( \beta_L \) Subunit**—We next explored the activation (tyrosine phosphorylation) of Jak1 and Tyk2 in cells expressing \( \beta_L \) chain truncated at amino acids 417, 346, or 300, respectively. Because the \( \beta_L \) subunit associates with Jak1 (see below), we used a GST fusion protein encoding the cytoplasmic domain of this chain to precipitate Jak1. Fig. 3 (center panel) shows that tyrosine phosphorylation of Jak1 is observed in cells expressing \( \beta_L \) subunit truncated at residue 417 or 346 but not when the \( \beta_L \) chain was truncated at amino acid 300 (\( \alpha \beta_L \)300). Stripping and reblotting of the filter with an anti-Jak1 antibody demonstrated that similar amounts of Jak1 were precipitated by GST\( \beta_L \) in all cell lines (Fig. 3, lower panel). Similar results were obtained when precipitations were performed with anti-Jak1 antibodies (data not shown).

A tyrosine-phosphorylated protein with slower electrophoretic mobility than Jak1 was precipitated in some experiments by GST\( \alpha \) and not by anti-Jak1 antibodies, in \( \alpha \beta_L \)346 cells (Fig. 3). The nature of this protein remains to be elucidated. We also studied tyrosine phosphorylation of Tyk2 in the different transfectants in response to IFN\( \alpha 2 \). Activation of Tyk2 was consistently detected in cells expressing \( \beta_L \) chain truncated at amino acids 417 and 346, and in some experiments very low levels of Tyk2 phosphorylation were also observed in \( \alpha \beta_L \)300 cells (data not shown). Murine IFN\( \beta \) was able to induce Jak kinase phosphorylation in all these stable transfectants (data not shown).

We also performed EMSA using probes for the IFN-stimulated response element and the m75SIE elements. These probes allowed us to study the activation of Stat1, Stat2, and Stat3 factors in response to human IFN\( \alpha 2 \) in L-929 transfectants carrying deletions of the cytoplasmic domain of the \( \beta_L \) chain. Fig. 4A (upper panel) shows that ISGF3 activation is induced by human IFN\( \alpha 2 \) (hu), human IFN\( \beta \) (hu\( \beta \)), and murine IFN\( \alpha \) (mu\( \alpha \)) in L-929 transfectants expressing \( \beta_L \) subunit truncated at residues 462 and 346 (lanes 2–4 and 6–8, respectively), but not when this chain is truncated at amino acid 300 (Fig. 4B, lane 2). However, ISGF3 is induced when \( \alpha \beta_L \)300 cells are treated with mouse IFN\( \alpha \) (Fig. 4C, lanes 2–4),

![Fig. 2. Characterization of L-929 transfectants expressing wild-type \( \alpha \) subunit and truncations of \( \beta_L \).](image-url)

**Table I**

| Transfectant | \( K_d \) Receptors/cell | \( K_d \) Receptors/cell |
|--------------|--------------------------|--------------------------|
| \( \alpha \) subunit | | |
| LpRZ\( \alpha L_{462} \) | 22 ± 7 | 2,645 ± 706 |
| LpRZ\( \alpha L_{417} \) | 27 ± 8 | 3,180 ± 492 |
| LpRZ\( \alpha L_{360} \) | 91 ± 65 | 2,412 ± 662 |
| LpRZ\( \alpha L_{346} \) | 38 ± 11 | 780 ± 220 |
| LpRZ\( \alpha L_{300} \) | 63 ± 1 | 4,680 ± 170 |

\( a \) The \( K_d \) and the number of sites/cell were calculated using the computer program Ligand. In all transfectants, the two-site model was statistically more significant than the one-site model (\( p < 0.05 \)). Competitive displacement of \( ^{125}I \)-labeled IFN\( \alpha 2 \) by unlabeled IFN\( \alpha 2 \) was performed as described previously (7).
indicating that the Stat pathway is functional in these cells. The ISGF3 complex is supershifted by an anti-mouse Stat2 serum (Fig. 4C, lane 3).

EMSA experiments performed with the m67SIE element present in the c-fos promoter showed that full activation of Stat1 and Stat3 also requires amino acids 265–346 of the cytoplasmic domain of the β_L chain (Fig. 4, A and B, lower panels, compare the induction of the different c-sis-inducible factor complexes in aβL360 and aβL346 cells). Low levels of IFNα2-induced activation of Stat1 and Stat3 were observed in aβL300,2 cells that are resistant to the antiviral effect of IFNα2 (Fig. 4B, lower panel, lane 2). The c-sis-inducible factor complexes containing Stat1 and Stat3 were induced by murine IFNαβ and supershifted by anti-Stat1 and anti-Stat3 serum (Fig. 4C, lanes 7 and 9).

The β_L Chain Specifically Associates with Jak1—To test for a possible interaction between the β subunit and Jak1, we produced GST fusion proteins encoding the entire cytoplasmic domain of the β_L and β_S chains. These GST fusion proteins were used to precipitate lysates from U-266 cells treated with IFNα2 or left untreated. Precipitates were resolved by SDS-PAGE and then subjected to immunoblotting with anti-Jak1 antibodies. Fig. 5A shows that Jak1 is precipitated with the β_L265–515 fusion protein and the control anti-Jak1 antibody before and after IFNα2 treatment (lanes 7 and 12). GSTβ_L (lane 2), along with antibodies against Stat1, Stat2, and Tyk2, failed to detect Jak1. To define the role of the Box 1 motif in the interaction between Jak1 and the β_L chain we produced two GST fusion proteins: (a) one containing only the first 34 amino acids (β_L265–298) of the cytoplasmic domain, which include the Box 1 motif (AAs 287–295); and (b) a second consisting of all the cytoplasmic residues except the first 34 amino acids (AAs 300–515), thus deleting the Box 1 region. Interestingly, the β_L265–298 construct precipitated Jak1 at levels that were barely above background (Fig. 5A, lane 4), whereas the 300–515 amino-terminal deletion, lacking the entire Box 1 domain, bound Jak1 roughly at the same level (80%) as the full-length protein (Fig. 5A, lanes 7 and 8). This result indicates that the binding of Jak1 to the β_L chain requires a region carboxyl-terminal to the Box 1 motif rather than Box 1 itself. To further define the Jak1 binding site, we produced GST fusion proteins encoding truncated forms of the cytoplasmic domain. GST fusion proteins encoding the β_L chain truncated at residues 462, 375, or 346 all bound Jak1 at comparable levels (Fig. 5A, lanes 3, 5, and 6), suggesting that the primary Jak1 binding site resides between residues 300–346.

To determine whether the interaction between Jak1 and the β_L chain could be detected in vivo, lysates obtained from U-266 cells treated with IFNα2 or left untreated were immunoprecipitated with antibodies against the different receptor subunits and Jak1. Fig. 5B shows an association between the

**Table II**

*Antiviral activity in L-929 cells transfected with α chain and truncated forms of the β_L subunit*

| Transfectant | Mouse IFNaβ<sup>α</sup> | Human IFNaβ<sup>α</sup> |
|-------------|-----------------|-----------------|
| LpRZαβ<sub>L462</sub> | 8 units/ml | 4 units/ml |
| LpRZαβ<sub>L247</sub> | 12 | 15 |
| LpRZαβ<sub>L360</sub> | 18 | 45 |
| LpRZαβ<sub>L346</sub> | 12 | 30 |
| LpRZαβ<sub>L300</sub> | 3 | >500 |

*Cytopathic effect assay was performed using a 1:25,000 dilution of encephalomyocarditis virus stock that produced 100% cytopathic effect in 24 h. The data shown represent the amount of the respective IFN L chain produced by the indicated subunits of the type I IFN-R were treated for 15 min with human IFNα2 or left untreated. Precipitations were performed with a GST fusion encoding the entire cytoplasmic domain except the first 34 amino acids (AAs 300–346). GST fusion proteins: (a) containing only the first 34 amino acids (β_L265–298) of the cytoplasmic domain, which include the Box 1 motif (AAs 287–295); and (b) a second consisting of all the cytoplasmic residues except the first 34 amino acids (AAs 300–515), thus deleting the Box 1 region. Interestingly, the β_L265–298 construct precipitated Jak1 at levels that were barely above background (Fig. 5A, lane 4), whereas the 300–515 amino-terminal deletion, lacking the entire Box 1 domain, bound Jak1 roughly at the same level (80%) as the full-length protein (Fig. 5A, lanes 7 and 8). This result indicates that the binding of Jak1 to the β_L chain requires a region carboxyl-terminal to the Box 1 motif rather than Box 1 itself. To further define the Jak1 binding site, we produced GST fusion proteins encoding truncated forms of the cytoplasmic domain. GST fusion proteins encoding the β_L chain truncated at residues 462, 375, or 346 all bound Jak1 at comparable levels (Fig. 5A, lanes 3, 5, and 6), suggesting that the primary Jak1 binding site resides between residues 300–346.

*Fig. 3. Activation of Jak1 kinase.* Mouse L-929 cells transfected with the indicated subunits of the type I IFN-R were treated for 15 min with human IFNα2 or left untreated. Precipitations were performed with a GST fusion encoding the entire cytoplasmic domain of β_L (β_L265–515) to detect Jak1. This GST fusion protein precipitates Jak1 as demonstrated in Fig. 5A but produces lower levels of background in anti-phosphotyro sine immuno blots. Immunoblotting was performed with the anti-phosphotyrosine mAb 4G10 (upper panel). Bottom panel, an anti-Jak1 mAb immuno blot of the same filter.

*Fig. 4. EMSA with ISGF3 and m67SIE probes.* A, whole cell extracts were obtained from mouse L-929 cells stably expressing wild-type α subunit and β_L chain truncated at amino acids 346 and 462 (lanes 1–4 and 5–8, respectively). Cells were treated with 10,000 units/ml murine IFNαβ (lanes 2 and 6), human IFNβ (lanes 3 and 7), and human IFNα2 (lanes 4 and 8) or left untreated (lanes 1 and 5) for 20 min at 37 °C. EMSA was performed using IFN-stimulated response element and m67SIE probes (upper panel and lower panels, respectively) (24). B, whole cell extracts were obtained from mouse L-929 cells stably expressing wild-type α subunit and β_L chain truncated at amino acids 300 or 346 (lanes 1 and 2 and lanes 3 and 4, respectively). Cells were treated with human IFNα2 as described in A. EMSA was performed using IFN-stimulated response element and m67SIE probes (upper panel and lower panels, respectively). C, aβL360 cells were treated with murine IFNαβ (+) or left untreated (−). Supershifts of the ISGF3 complex were performed with an anti-Stat2 serum (Santa Cruz Laboratories; lane 3) and with anti-Stat1 and anti-Stat3 sera for the c-sis-inducible factor complexes (lanes 7 and 9). Normal rabbit serum was used as a negative control (lanes 4 and 8). The migration of the different complexes is indicated.
The Jak1 protein was produced using a wheat germ translation kit, labeled with \([35S]methionine\), and precipitated with the appropriate GST fusion proteins or control antibodies, resolved by SDS-PAGE, transferred to polyvinylidene difluoride, and blotted with anti-Jak1 monoclonal antibody. The \(\beta_L\) subunit interacts with Jak1 in vitro. Cell lysates obtained from U-266 cells treated with 20,000 units/ml IFN\(a\) or left untreated were immunoprecipitated with 5 \(\mu\)l of sera directed toward the indicated regions of the receptor or normal rabbit serum (NR), transferred to polyvinylidene difluoride, and immunoblotted with an anti-Jak1 mAb (1:1000 dilution). C, direct interaction between the \(\beta_L\) subunit and Jak1. Jak1 was produced in an in vitro translation assay. Proteins were labeled with \([35S]methionine\) and then precipitated with the indicated GST fusion proteins or a Jak1 antibody. Precipitated proteins were resolved by SDS-PAGE, transferred to polyvinylidene difluoride, and exposed to x-ray film overnight. No tyrosine phosphorylation of Jak1 or the GST fusion proteins was detected when Jak1 was produced by this method. It is possible that Jak inhibitors are present in the cell-free system that may be required for full kinase activation (15–18, 26–34). In contrast, we have shown that Jak1 binding to the fusion protein containing the Box1 domain is necessary for activation of Jak1. For example, the minimal binding interaction between Jak1 and the fusion protein containing only Box1 may result from noncontiguous binding of Jak1 at both Box1 and AAs 300–346 sites or an extended Jak1-binding site that encompasses a region extending from the Box1 domain to AA 346 on the \(\beta_L\) subunit. This corresponds di- rectly with our in vitro data showing that cells expressing the Box1 construct, which contains the Box1 region (but excludes the major Jak1 binding site), do not activate Jak1 in the presence of IFN\(a\). Similarly, previous reports showed that mutation of residues in the Box 1 domain of the interleukin 2 receptor \(\beta\) chain do not significantly effect activation of Jak1 (35). Our data indicate that the primary Jak1-binding region corresponding to residues 300–346 on the \(\beta_L\) subunit is required for activation of the Jak-Stat pathway and induction of the antiviral response; however, it is possible that the Box 1 site is also necessary for activation of Jak1. For example, the minimal binding interaction between Jak1 and the fusion protein containing only Box 1 may result from noncontiguous binding of Jak1 at both Box 1 and AAs 300–346 sites or an extended Jak1-binding site that encompasses a region extending from Box 1 to AA 346 on the \(\beta_L\) subunit. The data presented herein for Jak1 and previous studies for Jak2 suggest that the homologous Jak kinases interact differently with their specific receptor subunits. For example, Jak2 requires the Box 1 motif for binding and, in addition, a less-conserved region distal to Box 1, designated as Box 2, that seems to be necessary in most cases for full kinase activation (15–18, 26–34). In contrast, we have observed that for the IFN\(\alpha\)R\(\beta\) chain, Jak1 binding and activation relies on a site carboxyl-terminal to Box 1. Similarly, an equivalent region designated in some cases as Box 2 (i.e., interleukin 2 receptor \(\beta\) chain) has been reported to be necessary for Jak1 activation by other cytokines (34–36). Experiments are currently under way to establish the role of the distal part of the \(\beta_L\) chain in IFN\(\alpha\) signaling.

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