COMMENTS

Hypothesis: Ligand/Receptor-Assisted Nuclear Translocation of STATs (44282)

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Abstract. **The STAT transcription factors are mediators of signal transduction of a variety of factors, including interferons (IFNs), interleukins, growth factors, and peptide hormones. Subsequent to activation, STATs are translocated to the nucleus apparently through the well-described importin/Ran system, where they activate target genes. Molecules utilizing this nuclear import system require specific nuclear localization sequences (NLSs). Paradoxically, such NLSs are not identifiable on STATs, thus raising the question of how they are imported into the nucleus. Of considerable interest is the observation that ligands and/or receptors that signal through STATs contain putative NLSs and, where examined, either ligand or receptor undergoes nuclear translocation. We hypothesize that ligands and/or their receptors serve as vehicles for the nuclear translocation of STATs, and that they may be directly involved in signal transduction. Using IFN_γ as a model system, we provide a possible mechanism for how this direct role is fulfilled. A functional NLS has been identified in a C-terminal domain of IFN_γ. This domain and the NLS contained within are crucial for the biological properties of IFN_γ in that a peptide encompassing this domain is sufficient to induce an antiviral state. Further, this domain binds specifically to a membrane-proximal region internal cytoplasmic domain of the α subunit of the receptor complex in a region that is directly involved in the recruitment and activation of the JAK/STAT pathway. We suggest that this novel mode of receptor recognition and activation may be a driving force for nuclear translocation of molecules like STATs that are associated with the ligand-receptor complex.** [P.S.E.B.M. 1998, Vol 218]

During the last several years, a family of transcription factors called signal transducers and activators of transcription (STATs) have been identified and characterized (reviewed in Ref. 1). STATs have been shown to serve as transcription factors for interferons (IFNs), other cytokines, and growth factors. STATs, which range in molecular weight from 84 to 113 kD and are constitutively expressed in the cytoplasm, translocate to the nucleus only upon activation by an extracellular signal. The size of the STATs further increases since various homo- or heterocomplexes are formed upon activation. Based on current knowledge, transcription factors of this size must enter the nucleus *via* active transport through the nuclear pore complex (NPC) (2).

In general, the process of active transport through the NPC occurs *via* interaction with proteins that bind to a transcription factor (or other protein destined for the nucleus) and dock at the NPC (Fig. 1) (reviewed in Ref. 2). Transcription factors bind to a heterocomplex called importin, which is made up of α and β subunits (importin α and β, respectively). The transcription factor binds to importin α *via* a specific motif called the nuclear localization sequence

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Figure 1. Working model for ligand (IFN_γ) binding to receptor and translocation of STAT transcription factors to the nucleus. Our preliminary data demonstrate the binding of IFN_γ *via* the C-terminus to the cytoplasmic domain of the receptor, and enhance JAK binding. This, in turn, causes STAT binding. IFN_γ provides the NLS for translocation to the nucleus since none of the other participants have been shown to possess an NLS. α = importin α; β = importin β; GAS = gamma-activated sequence.

(NLS). NLSs are short amino acid stretches characterized by clusters of polycationic residues occurring together as a single group or two short groups separated by a fixed spacer (bipartite NLS sequences; 3). Following the binding of the NLS-bearing protein, importin α interacts with importin β through its importin β binding domain. Importin β mediates docking of the complex at the NPC. Translocation into the nucleus requires an NPC-associated GTPase called Ran, as well as other co-factors. Thus, transcription factors enter the nucleus *via* an energy-requiring mechanism.

Recently it was shown that the GTPase activity of both the Ran/TC4 and the NPC are required for nuclear transport of IFN_γ-activated STAT1 (4). Thus, some if not all, STATs appear to use the nuclear transport mechanism depicted in Figure 1. However, based on current knowledge there is a potential paradox. STATs, like STAT1 for example, do not contain a polycationic NLS (4). As a rule, NLS-containing proteins are synthesized in the cytoplasm and appear to be constitutively transported to the nucleus immediately thereafter. So how is it that preformed cytoplasmic proteins such as the STATs, which require nuclear import for their functions, are actively translocated in the absence of an NLS? It seems reasonable to assume that other NLS-containing molecules must associate in some fashion with STATs in order to use the NPC machinery for transportation to the nucleus. The precedence for the presence of NLS-containing nuclear chaperone proteins assisting in the bind of cytoplasmic non-NLS proteins to importin α has recently been established in the case of the human aryl hydrocarbon receptor (5).

It has been demonstrated by several investigators that IFN_γ, which activates STAT1, rapidly translocates to the nucleus (6, 7). The C-terminus of IFN_γ contains a putative NLS (RKRKRSR) that is very similar to that found in the SV40 large T antigen. This region has been referred to as

the polycationic tail of IFN_γ. This particular sequence has been shown to be essential for the biological activity of IFN_γ, although no clear function has been ascribed to it to date (8–10). Related to this, at least three independent lines of experimentation have identified intracellular IFN_γ as being biologically active: 1) human IFN_γ (huIFN$_\gamma$) delivered by a liposome vector was able to activate murine macrophages to a tumoricidal state; 2) secretion-defective huIFN$_\gamma$ expressed in murine fibroblasts triggered antiviral activity; and 3) microinjected IFN_γ induced Ia expression on murine macrophages (11–13). The activity of huIFN$_\gamma$ in these murine cells defies the well-known species-specificity of exogenously supplied huIFN$_\gamma$ that does not have activity on murine cells. Thus, the IFN_γ molecule most likely interacts with some intracellular element(s) to induce a biologic response. Similarly, intracellularly expressed IFN_α has also been shown to be biologically active and can activate the DNA binding activity of the ISGF3 transcription complex of which STAT1 and STAT2 are integral components (14). The formation of an active ISGF3 complex involves the translocation of both STAT1 and STAT2 to the nucleus. Thus, it seems likely that one of the reasons that IFN_γ is active intracellularly is because it can activate STAT1.

We hypothesize that a key intracellular interaction that comes into play in bringing about these effects of IFN_γ is its interaction with the nuclear transport mechanism in an NLS-dependent manner. Our recent studies, which are described below, demonstrate that the polycationic tail of IFN_γ does indeed contain a functional NLS that is capable of transporting a heterologous protein to the nucleus when coupled to it in a manner analogous to that for the NLS of the SV40 large T antigen. Thus, the translocation of IFN_γ itself to the nucleus is mediated by an NLS sequence residing in the polycationic tail, which apparently is crucial for the biological function of the ligand. Given that intracellular IFN_γ 1) is biologically active; 2) can activate transcription factors like STATs that do not themselves appear to contain an NLS for nuclear translocation; and 3) contains an NLS competent for nuclear translocation, we suggest that IFN_γ functions intracellularly as a nuclear chaperone protein that assists in the binding and translocation of signaling molecules like the STATs *via* the importin/Ran machinery. Further, this proposed mechanism may not be limited to cytokines such as the IFNs, but may also be a means by which hormones such as growth hormone (GH) exert their effects intracellularly.

Over the last few years, our studies on IFN_γ binding to its receptor have provided insight into a unique mode by which IFN_γ can interact directly with intracellular signaling elements *via* the cytoplasmic domain of the α chain of the IFN_γ receptor complex. The studies outlined below provide several scenarios in which to examine in greater detail the intracellular role of IFN_γ in signaling by transcription factors like STATs, including the question of maintaining the specificity of signal transduction unique to any given stimulatory ligand.

Interaction of IFN_γ with the IFN_γ Receptor ($IFN_\gamma R$)

Previously, we showed, using intact cells, that IFN_γ binds to the extracellular domain of the $IFN_\gamma R$ α chain through its N-terminus (15–18). Using synthetic peptides, we showed that an N-terminus peptide consisting of residues 1–39, IFN_γ(1–39), blocked both IFN_γ binding to receptor and IFN_γ antiviral activity (12–15). Other peptides encompassing the remainder of the IFN_γ sequence had no effect on binding.

The peptide studies were then repeated using a soluble murine $IFN_\gamma R$ construct consisting of both the extracellular domain and intracellular domain (19). Competition for receptor binding by the N-terminus peptide was again observed under these conditions. In this case, however, a murine C-terminus IFN_γ peptide consisting of residues 95–133, IFN_γ(95–133), was also able to compete with IFN_γ for receptor (20). In combination, the N-terminus and C-terminus peptides were more competitive than either peptide alone, suggesting that the peptides recognized different sites on the $IFN_\gamma R$ that resided separately in the extracellular and intracellular domains of the receptor α chain.

Further, through the use of synthetic receptor peptides, we showed that the N-terminus of IFN_γ recognized the extracellular domain of the $IFN_\gamma R$ α chain, whereas the C-terminus recognized the cytoplasmic domain of the receptor (21). Recently, we have cloned and expressed the entire cytoplasmic domain of the human $IFN_\gamma R$ α chain independent of a fusion protein (22). Radiolabeled human IFN_γ (^{125}I-IFN_γ) bound to the cytoplasmic $IFN_\gamma R$ domain, and this binding was inhibited by intact human IFN_γ and C-terminus peptide, whereas the N-terminus peptide had no effect. We have shown previously that the N-terminus peptide blocks extracellular receptor binding, whereas the C-terminus peptide is not inhibitory (15–18). Thus, the above data show that IFN_γ recognizes the $IFN_\gamma R$ α chain extracellular domain *via* its N-terminus, and the cytoplasmic domain *via* its C-terminus.

Interestingly, a C-terminus IFN_γ peptide that binds to the cytoplasmic domain of the receptor and that contains the NLS has biological activity (23). Treatment of murine macrophage cell lines with mouse IFN_γ(95–133), where the peptide is taken up by pinocytosis, results in 10-fold upregulation of MHC class II molecule expression and 10^6–10^9-fold increased resistance to viral infection. Further, a C-terminus peptide corresponding to human IFN_γ(95–134) also has activity in murine macrophage cells. These data suggest a direct role for the C-terminus of IFN_γ in the initiation of intracellular signaling, probably related to its nuclear translocation properties.

To this end we have coupled R-phycoerythrin (PE), an autofluorescent protein, to the C-terminal IFN_γ peptide,

IFN_γ(95–132), which contains the putative NLS. Standard *in vitro* transport assays using rabbit reticulocyte lysate were carried out in mouse 3T3 cells clone A31, permeabilized with digitonin (24). As can be seen in Figure 2A, the IFN_γ(95–132)-PE complex underwent nuclear translocation. In contrast, PE failed to undergo nuclear translocation when added to the cells with IFN_γ(95–132) under conditions where the two were not covalently complexed (Figure 2B; Subramaniam *et al.*, unpublished observations). These findings provide crucial evidence that the C-terminus of IFN_γ contains a functional NLS that is capable of transporting heterologous proteins to the nucleus, and also provide an important starting point for further examining the hypothetical model presented in Figure 1.

Recently, the crystal structure of human IFN_γ complexed to the extracellular domain of the α chain receptor subunit was determined (25). The obvious question is how do the peptide and related studies fit with the x-ray structure data? IFN_γ bound to the extracellular domain of the receptor *via* its N-terminus, consisting of a helix-loop-helix structure. This secondary structure is contained in the N-terminus peptide IFN_γ(1–39) as determined by two-dimensional NMR (26). The C-terminus of IFN_γ did not bind to the extracellular domain of the receptor, which is again consistent with our peptide studies. Thus, the x-ray structure data are in agreement with peptide studies in identifying the sites of interaction between IFN_γ and the receptor α chain. Based on the peptide studies, which have been very consistent with the structure data on the extracellular receptor domain, one would predict that further x-ray structure studies would show that IFN_γ interacts with the cytoplasmic domain of the receptor *via* the C-terminus of IFN_γ.

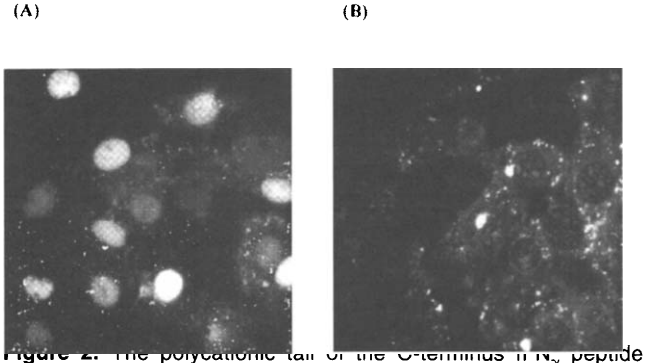

Figure 2. The polycationic tail of the C-terminus of IFN_γ peptide IFN_γ(95–132) is a nuclear localization sequence. Standard *in vitro* transport assays using rabbit reticulocyte lysate were carried out in mouse 3T3 cells (clone A31) permeabilized with digitonin using previously described procedures (62). The C-terminus peptide was covalently coupled to R-phycoerythrin (PE) through the bifunctional coupling agent sulfo-SMCC (Pierce, Rockford, IL) and used as the substrate. (A) Nuclear uptake of IFN_γ(95–132)-PE complex. Although the cells showed variations in nuclear staining, typically up to 85% of cells in any given field showed nuclear transport. Note the morphological outline of the nucleus including the nucleoli. (B) Failure of nuclear uptake of a simple mixture of IFN_γ(95–132) and PE where the two were not covalently coupled. Note the presence of some PE in the cytoplasm but with its conspicuous absence from the nucleus.

The cytoplasmic domain of the IFN_γ receptor α chain is obligatorily required for IFN_γ-induced biologic responses and, surprisingly, is not species-restricted. The region of the cytoplasmic domain that interacts with IFN_γ encompasses residues 252–291. In the human receptor, this region contains a membrane proximal leucine-isoleucine (LI) sequence (residues 270–271) that is required for receptor trafficking events within the cell following receptor endocytosis (27). Immediately upstream of this sequence is an LPKS sequence (residues 266–269) that is required for the constitutive association of the tyrosine kinase JAK1 (28). This association is crucial for the activation of the signal transduction cascade from the receptor following binding of IFN_γ. In the murine receptor, we have also shown that the cytoplasmic domain of the α chain also contains two sites of interaction for the other tyrosine kinase, JAK2 (29). One site encompassing residues 283–209 in the murine receptor overlaps the binding site for the C-terminus of IFN_γ. In particular, binding of JAK2 at this site on the receptor is enhanced by interaction of the C-terminus of IFN_γ with the cytoplasmic domain, which may explain why co-precipitation of JAK2 with the α chain is seen only after ligand treatment of cells (30). Given the proximity of the ligand-induced JAK2 binding site to binding sites for IFN_γ and JAK1 on the cytoplasmic domain, it is quite probable that the interaction of the ligand with this domain of the receptor directly contributes to the initial activation of either kinases and consequently initiation of the signal transduction cascade. Finally, phosphorylation of the tyrosine at position 440 generates a binding site on the receptor for STAT1. This site overlaps the second interaction site for JAK2 on the α chain. Thus, interaction of the NLS-bearing C-terminus of the ligand with the cytoplasmic domain of the receptor could affect processes that include the activation of the signal events that recruit STAT transcription factors within the ligand-receptor complex and the subsequent endocytosis, trafficking, and nuclear translocation of these molecules to their target genes.

Putative NLSs in Other STAT-Utilizing Proteins

As indicated earlier, IFN_γ is not the only protein that uses the STAT signaling pathway. If a ligand and/or receptor NLS is involved in the nuclear translocation of STATs, then one would expect to find putative NLS motifs associated with other STAT-utilizing ligands and/or their receptors. As shown in Table I, this is indeed the case. It would seem more than just coincidence that diverse ligands such as insulin, IFNs, colony stimulating factors, and nonhematopoietic growth factors also possess an NLS and use the JAK/STAT signal transduction pathway in gene activation. In fact, it is suggestive that NLS-bearing ligands and/or their receptors play a direct role in nuclear translocation of transcription factors such as the STATs. Further, ligands such as insulin, growth hormone (GH), and IL-1, undergo nuclear

Table I. Putative Nuclear Localization Sequences (NLS) Found on Some STAT-Utilizing Ligands

Factor	NLS Motif[a]	NLS found on	References
hPDGFA	PRESGKKRKRKR	ligand	31, 1
hPDGFB	RVTIRTVRVRRPPKGKHRK	ligand	31, 1
hIFNα	QKRLRRKD	ligand (IFNαConsensus)	32, 1
	RKIIEKKT	receptor (α-chain)	33, 1
mIFNγ	LRKRKRSRC	ligand	34,1
hIFNγ	NSNKKKR	ligand	35,1
	KTGKRKRS	ligand	35, 1
hIL-1α	KVLKKRRL	ligand	36, 37
hIL-1β	KKKMEKRF	ligand	36, 37
mIL-1	NVKKSRRL	receptor	38, 37
hIL-2	QRRQRKSRR	receptor (α-chain)	39, 40
mIL-3	RKSLLYRLCPPIPRLR	receptor (α-chain)	41, 42
mIL-4	KHRVKKK	receptor	43, 1
hIL-4	HRHKQLIRFLKRLER	ligand	44, 1
mIL-5	KKYIDRQKEKCGEERRRTR	ligand	45, 42
hIL-5	KKYIDQQKKKCGEERRRV	ligand	46, 47
mIL-6	KKPCPDDLKSVDLFKKEK	receptor (gp 130)	48, 1
hIL-6	KKPF PEDLKS LDLFKKEK	receptor (gp130)	49, 1
mIL-7	KKKYLKKVKHD	receptor	50, 51
mIL-7	KKRIKPVVWPSLPDHKK	receptor	50, 51
hIL-7	KKRIKP I VWPSLPDHKK	receptor	50,51
hIL-10	RRRKKLPSVLLFKK	receptor	52, 53
mIL-11	KKPCPDDLKSVDLFKKEK	receptor (gp130)	48, 54
hIL-11	KKPF PEDLKS LDLFKKEK	receptor (gp130)	49, 54
LIF	KKPCPDDLKSVDLFKKEK	receptor (gp130)	49, 1
	KKPF PEDLKS LDLFKKEK	receptor (gp130)	49, 1
OSM	KKPCPDDLKSVDLFKKEK	receptor (gp130)	49, 1
	KKPF PEDLKS LDLFKKEK	receptor (gp130)	49, 1
CNTF	KKPCPDDLKSVDLFKKEK	receptor (gp130)	49, 1
	KKPF PEDLKS LDLFKKEK	receptor (gp130)	49, 1
mInsulin	RRSYALVSLSFFRKLH	receptor	55, 56
hInsulin	RRSYALVSLSFFRKLR	receptor	57, 56
hGH	VRVRSKQRN	receptor	58, 1
hEGF	RRRHIVRKRTLRRL	receptor	59, 1
hGM-CSF	RNSKRRREIR	receptor	60, 42

[a] These putative NLSs have been identified by comparison with protein sequences known to have nuclear localization function. A partial list of these sequences has previously been published (see reference 64).

translocation (61–63). The studies on GH internalization and nuclear translocation, for example, provide some interesting insights (61). Internalization requires a full-length receptor since no internalization occurred in cells transfected with GH receptor lacking the cytoplasmic domain. Once internalized, GH rapidly translocated to the nucleus. An apparent anomaly in the nuclear translocation of GH is its lack of an NLS, suggesting that it must associate with another NLS-bearing molecule in order to localize to the nucleus. Proteins that have been shown to interact with GH intracellularly, such as the GH binding protein, are not required for nuclear localization (61). However, the GH receptor possesses a putative NLS (Table I), suggesting that it may be involved in the translocation of GH to the nucleus. Electron microscopic analysis indicates that GH interacts with the NPC at the nuclear membrane prior to entering the nuclear matrix. Thus, GH binds to a full-length receptor and is internalized, it then translocates to the nucleus utilizing the NPC, possibly *via* the putative NLS on the receptor.

A Model for Nuclear Translocation of STATs

A model depicting the direct involvement of IFN_γ in nuclear translocation of its transcription factor STAT1 is presented in Figure 1. IFN_γ binds to receptor *via* its N-terminus and undergoes endocytosis. This is followed by IFN_γ binding to the cytoplasmic domain of the receptor *via* its C-terminus, which contains a putative NLS. STATs do not contain putative NLSs, so IFN_γ provides the NLS for the translocation of the complex of IFN_γ, receptor, and STAT to the nucleus. In this manner, STAT translocation to the nucleus can occur in the absence of an NLS within its sequence. Key to this model are data presented in Figure 2, which show that the putative IFN_γ NLS does indeed cause nuclear translocation of proteins.

The translocation of the ligand and/or receptor complex to the nucleus would obviously confer an added layer of specificity especially in a scenario when multiple ligands appear to activate the same STAT molecules but turn on

different sets of genes. STAT activation appears to involve recruitment and binding to phosphotyrosine residues on the cytoplasmic domains of the signal transducing receptor. The direct translocation of a ligand-receptor complex bearing the activated transcription factor to the nucleus would in essence sequester and compartmentalize a portion of the STAT pool by the ligand-receptor pair generating the signal. Further, the discrimination between similar STAT-responsive elements within multiple promoters could in principle be directed by ligand- or receptor-specific secondary interactions with other elements of the transcription machinery. Inherent in this proposition is the fact that a more important function is ultimately served by this translocation. This would be to shuttle to the nucleus signaling molecules associated with the complex that do not have an NLS to target them for nuclear transport but require nuclear import for function. Thus, it is proposed that the translocation of such signaling molecules to the nucleus is obligatorily linked to the translocation of ligand and/or receptor that transmits the signal. The presence of putative NLSs on ligands and/or receptors has been well noted by others and has been suggested to be the mechanism by which ligands and/or receptors translocate to the nucleus (64). However, linkage of this concept to the nuclear translocation of transcription factors or other proteins such as the STATs that do not possess an NLS has not been proposed. Moreover, our studies on the binding of the C-terminus of IFN_γ to the cytoplasmic domain of the receptor suggest a starting point for the development of a model for nuclear translocation of transcription factors, such as the STATs, that interact with the cytoplasmic domain of receptors like that of IFN_γ.

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1. Leaman DW, Leung S, Li X, Stark GR. Regulation of STAT-dependent pathways by growth factors and cytokines. FASEB J **10:**1578–1588, 1996.
2. Gorlich D, Mattaj IW. Nucleocytoplasmic transport. Science **271:**1513–1518, 1996.
3. Dingwall C, Laskey RA. Nuclear targeting sequences: A consensus? Trends Biochem Sci **16:**478–481, 1991.
4. Sekimoto T, Nakajima K, Tachibana T, Hirano T, Yoneda Y. Interferon-γ-dependent nuclear import of STAT1 is mediated by the GTPase activity of Ran/TC4. J Biol Chem **271:**31017–31020, 1996.
5. Eguchi H, Ikuta T, Tachibana T, Yoneda Y, Kawajiri K. A nuclear localization signal of human aryl hydrocarbon translocation/hypoxia-inducible factor 1β is a novel bipartite type recognized by the two components of nuclear pore-targeting complex. J Biol Chem **272:**17640–17647, 1997.
6. MacDonald HS, Kushnaryov VM, Sedmak JJ, Grossberg SE. Transport of γ-interferon into the cell nucleus may be mediated by nuclear membrane receptors. Biochem Biophys Res Commun **138:**254–260, 1986.
7. Bader T, Wietzerbin J. Nuclear accumulation of interferon γ. Proc Natl Acad Sci USA **91:**11831–11835, 1994.
8. Wetzel R, Perry LJ, Veilleux C, Chang G. Mutational analysis of the C-terminus of human interferon-γ. Protein Eng **3:**611–623, 1990.
9. Lundell D, Lunn C, Dalgarno D, Fossetta J, Greenberg R, Reim R, Grace M, Narula S. The carboxy-terminal region of human interferon-γ is important for biological activity: Mutagenic and NMR analysis. Protein Eng **4:**335–341, 1991.
10. Dobeli H, Gentz R, Jucker W, Garotta G, Hartmann DW, Hochuli E. Role of the carboxy-terminal sequence on the biological activity of human interferon (IFN-γ). J Biotechnol **7:**199–216, 1988.
11. Fidler IJ, Fogler WE, Kleinerman ES, Saiki I. Abrogation of species specificity for activation of tumoricidal properties in macrophages by recombinant mouse or human interferon-γ encapsulated in liposomes. J Immunol **135:**4289–4296, 1985.
12. Sanceau J, Sondermeyer P, Beranger F, Falcoff R, Vaquero C. Intracellular human interferon-γ triggers an antiviral state in transformed murine L cells. Proc Natl Acad Sci USA **84:**2906–2910, 1987.
13. Smith MR, Muegge K, Keller JR, Kung H-F, Young HA, Durum SK. Direct evidence for an intracellular role for IFN_γ. J Immunol **144:**1777–1782, 1990.
14. Rutherford MN, Kumar A, Coulombe B, Skup D, Carver DH, Williams BRG. Expression of intracellular interferon constitutively activates ISGF3 and confers resistance to EMC viral infection. J Interferon Cytokine Res **16:**507–510, 1996.
15. Russell JK, Hayes MP, Carter JM, Torres BA, Dunn BM, Russell SW, Johnson HM. Epitope and functional specificity of monoclonal antibodies to mouse interferon-γ. The synthetic peptide approach. J Immunol **136:**3324–3328, 1986.
16. Russell JK, Magazine HI, Torres BA, Johnson HM. Steric relationship of amino-terminal and carboxy-terminal domains of murine interferon-γ as assessed by monoclonal antibodies. J Interferon Cytokine Res **8:**433–439, 1988.
17. Jarpe MA, Johnson HM. Structure of an epitope in an immunodominant region of the interferon-γ molecule that is involved in receptor interaction. J Interferon Cytokine Res **10:**243–252, 1990.
18. Jarpe MA, Johnson HM. Topology of receptor binding domains of mouse IFN_γ. J Immunol **145:**3304–3309, 1990.
19. Griggs ND, Jarpe MA, Pace JL, Russell SW, Johnson HM. The N-terminus and C-terminus of IFN_γ are binding domains for cloned soluble IFN_γ receptor. J Immunol **149:**517–520, 1992.
20. Szente BE, Johnson HM. Binding of IFN_γ and its C-terminal peptide to a cytoplasmic domain of its receptor that is essential for function. Biochem Biophys Res Commun **201:**215–221, 1994.
21. VanVolkenburg MA, Griggs ND, Jarpe MA, Pace JL, Russell SW, Johnson HM. Binding site on the murine IFN_γ receptor for IFN_γ has been identified using the synthetic peptide approach. J Immunol **151:**6206–6213, 1993.
22. Green MM, Larkin J, Subramaniam PS, Szente BE, Johnson HM. Human IFN_γ receptor cytoplasmic domain: Expression and interaction with $HuIFN_\gamma$. Biochem Biophys Res Commun **243:**170–176, 1998.
23. Szente BE, Soos JM, Johnson HM. The C-terminus of IFN_γ is sufficient for intracellular function. Biochem Biophys Res Commun **203:**1645–1654, 1994.
24. Adam SA, Marr RS, Gerace L. Nuclear protein import in permeabilized mammalian cells requires soluble cytoplasmic factors. J Cell Biol **111:**807–816, 1990.
25. Walter MR, Windsor WT, Nagabhushan TL, Lundell DJ, Lunn CA, Zauodny PJ, Narula SK. Crystal structure of a complex between interferon-γ and its soluble high affinity receptor. Nature **376:**230–235, 1995.
26. Sakai TT, Jablonsky MJ, DeMuth PA, Krishna NR, Jarpe MA, Johnson HM. Proton NMR sequence specific assignments and secondary structure of a receptor binding domain of mouse γ-interferon. Biochemistry **32:**5650–5655, 1993.
27. Schreiber RD, Aguet M. The interferon-γ receptor. In: Nicola NA, Ed. Guidebook to Cytokines and their Receptors. Oxford: Oxford University Press, p120, 1994.
28. Kaplan DH, Greenlund AC, Tanner JW, Shaw AS, Schreiber RD. Identification of an interferon-γ receptor α chain sequence required for JAK1 binding. J Biol Chem **271:**9–12, 1996.
29. Szente BE, Subramaniam PS, Johnson HM. Identification of IFN_γ

receptor binding sites for JAK1 and enhancement by binding by IFN_γ and its C-terminal peptide IFN_γ(95–133). J Immunol **155:**5617–5622, 1995.
30. Kotenko SV, Izotova LS, Pollack BP, Mariano TM, Donelly RJ, Muthukumaran G, Cook JR, Garotta G, Silvennoinen O, Ihle JN, Pestka S. Interaction between the components of interferon-γ receptor complex. J Biol Chem **270:**20915–20921, 1995.
31. Deuel TF, Kawahara RS. Platelet-derived growth factor. In: Aggarwal BB, Gutterman JU, Eds. Human Cytokines: Handbook for Basic Research. Boston: Blackwell Scientific Publications, p300, 1992.
32. Pestka S. Interferon-α. In: Aggarwal BB, Gutterman JU, Eds. Human Cytokines: Handbook for Basic Research. Boston, Blackwell Scientific Publications, p3, 1992.
33. Uze G, Lutfalla G, Gresser I. Genetic transfer of a functional human interferon alpha receptor into mouse cells: Cloning and expression of its cDNA. Cell **60:**225–234, 1990.
34. Gray PW, Goeddel DV. Cloning and expression of murine immune interferon cDNA. Proc Natl Acad Sci USA **80:**5842–5846, 1983.
35. Gray PW. Interferon-γ. In: Aggarwal BB, Gutterman JU, Eds. Human Cytokines: Handbook for Basic Research. Boston: Blackwell Scientific Publications, p30, 1992.
36. Dower SK. Interleukin-1. In: Aggarwal BB, Gutterman JU, Eds. Human Cytokines: Handbook for Basic Research. Boston: Blackwell Scientific Publications, p46, 1992.
37. Tsukada J, Waterman WR, Koyama Y, Webb AC, Auron PE. A novel STAT-like factor mediates lipopolysaccharide, interleukin 1 (IL 1), and IL 6 signaling and recognizes a gamma interferon activation site–like element in the IL1B gene. Mol Cell Biol **16:**2183–2194, 1996.
38. Sims JE, March CJ Cosman D, Widmer MB, McDonald HR, McMahan CJ, Grubin CE, Wignall JM, Jackson JL, Call SM, Friend D, Alpert AR, Gillis S, Urdal DL, Dower SK. cDNA expression cloning of the IL-1 receptor, a member of the immunoglobulin superfamily. Science **241:**585–589, 1988.
39. Ishida N, Kanamori H, Noma T, Nikaido T, Sabe H, Suzuki N, Shimizu A, Honjo T. Molecular cloning and structure of the human interleukin-2 receptor gene. Nucleic Acids Res **13:**7579–7589, 1985.
40. Zhnag Q, Nowak I, Vonderheid EC, Rook AH, Kadin ME, Nowell PC, Shaw LM, Wasik MA. Activation of JAK/STAT proteins involved in signal transduction pathway mediated by receptor for interleukin-2 in malignant T lymphocytes derived from cutaneous anaplastic large T-cell lymphoma and Sezary syndrome. Proc Natl Acad Sci USA **93:**9148–9153, 1996.
41. Gorman DM, Itoh N, Kitamura T, Schreurs J, Yonehara S, Yahara I, Arai K, Miyajima A. Cloning and expression of a gene encoding an interleukin-3-receptor–like protein: Identification of another member of the cytokine receptor family. Proc Natl Acad Sci USA **87:**5459–5463, 1990.
42. Mui AL-F, Wakao H, O'Farrell AM, Harada N, Miyajima A. Interleukin-3, granulocyte-macrophage colony stimulating factor and interleukin-5 transduce signals through two STAT5 homologs. EMBO J **14:**1166–1175, 1995.
43. Mosley B, Beckman MP, March CJ, Idzerda RL, Gimpel SD, VandenBos T, Friend D, Alpert A, Anderson D, Jackson J, Wignall JM, Smith C, Gallis B, Sims JE, Urdal D, Widmer MB, Cosman D, Park LS. The murine interleukin-4 receptor: Molecular cloning and characterization of secreted and membrane-bound forms. Cell **59:**335–348, 1989.
44. Arai N, Nomura D, Villaret D, Malefijt RD, Seiki M, Yoshida M, Minoshima S, Fukuyama R, Maekawa M, Kudoh J, Shimizu N, Yokota K, Abe E, Yokota T, Takebe Y, Arai K. Complete nucleotide sequence of the chromosomal gene for human IL-4 and its expression. J Immunol **142:**274–282, 1989.
45. Misuta TR, Tanabe T, Nakakubo H, Noma T, Honjo T. Molecular cloning and structure of the mouse interleukin-5 gene. Growth Factors **1:**51–57, 1988.
46. Azuma C, Tanabe T, Konishi M, Kinashi T, Noma T, Matsuda F, Yaoita Y, Takatsu K, Hammarstrom L, Smith CIE, Severinson E, Honjo T. Cloning of cDNA for human T-cell replacing factor (interleukin-5) and comparison with the murine homologue. Nucleic Acids Res **14:**9149–9158, 1986.
47. Pazdrak K, Stafford S, Alam R. The activation of the JAK/STAT1 signaling pathway by IL-5 in eosinophils. J Immunol **155:**397–402, 1995.
48. Saito M, Yoshida K, Hibi M, Taga T, Kishimoto T. Molecular cloning of a murine IL-6-receptor–associated signal transducer, gp130, and its regulated expression *in vivo.* J Immunol **148:**4066–4071, 1992.
49. Hibi M, Murakami M, Saito M, Hirano T, Taga T, Kishimoto T. Molecular cloning and expression of an IL-6 signal transducer, gp130. Cell **63:**1149–1157, 1990.
50. Goodwin RG, Friend D, Ziegler SF, Jerzy R, Falk BA, Gimpel S, Cosman D, Dower SK, March CJ, Namen AE, Park LS. Cloning of the human and murine interleukin-7 receptors: Demonstration of a soluble form and homology to a new receptor superfamily. Cell **60:**941–951, 1990.
51. van der Plas DC, Smiers F, Pouwels K, Hoefsloot LH, Lowenberg B, Touw IP. Interleukin-7 signaling in human B cell precursor acute lymphoblastic leukemia cells and murine BAF3 cells involves activation of STAT1 and STAT5 mediated *via* the interleukin-7 receptor alpha chain. Leukemia **10:**1317–1325, 1996.
52. Liu Y, Wei SHY, Ho ASY, de Waal Malefyt R, Moore KW. Expression cloning and characterization of a human IL-10 receptor. J Immunol **152:**1821–1829, 1994.
53. Wehinger J, Gouilleux F, Groner B, Finke J, Mertelsmann R, Weber-Nordt RM. IL-10 induces DNA binding activity of three STAT proteins (Stat1, Stat3, and Stat5) and their distinct combinatorial assembly in the promoters of selected genes. FEBS Lett **394:**365–370, 1996.
54. Symes AJ, Rao MS, Lewis SE, Landis SC, Hyman E, Kink JS. Ciliary neutrotrophic factor coordinately activates transcription of neuropeptide genes in a neuroblastoma cell line. Proc Natl Acad Sci USA **90:**572–576, 1993.
55. Sibley E, Kastelic T, Kelly TJ, Lane MD. Characterization of the mouse insulin receptor gene promoter. Proc Natl Acad Sci USA **86:**9732–9736, 1989.
56. Chuang LM, Wang PH, Chang HM, Lee SC. Novel pathway of insulin signaling involving STAT1-alpha in Hep3B cells. Biochem Biophys Res Commun **235:**317–320, 1997.
57. Kenner KA, Kusari J, Heidenreich KA. cDNA sequence analysis of the human brain insulin receptor. Biochem Biophys Res Commun **217:**304–312, 1995.
58. Leung DW, Spencer SA, Cachianes G, Hammonds RG, Collins C, Henzel WJ, Barnard R, Waters MJ, Wood WI. Growth hormone receptor and serum binding protein: Purification, cloning, and expression. Nature **330:**537–543, 1987.
59. Das M, Manthrasalam R, Samanta A. Epidermal growth factor. In: Aggarwal BB, Gutterman JU, Eds. Human Cytokines: Handbook for Basic Research. Boston: Blackwell Scientific Publications, p365, 1992.
60. Crosier PS, Garnick MB, Clark SC. Granulocyte-macrophage colony-stimulating factor. In: Aggarwal BB, Gutterman JU, Eds. Human Cytokines: Handbook for Basic Research. Boston: Blackwell Scientific Publications, p238, 1992.
61. Lobie PE, Mertni H, Morel G, Morales-Bustos O, Norstedt G, Waters MJ. Receptor-mediated nuclear translocation of growth hormone. J Biol Chem **269:**21330–21339, 1994.
62. Podlecki DA, Smith RM, Kao M, Tsai P, Hueckteadt T, Brandenburg D, Lasher RS, Jarett L, Olefsky JM. Nuclear translocation of the insulin receptor: A possible mediator of the insulin receptor. J Biol Chem **262:**3362–3368, 1987.
63. Grenfell S, Smithers N, Miller K, Solari R. Receptor-mediated endocytosis and nuclear transport of human interleukin 1α. Biochem J **264:**813–822, 1989.
64. Jans DA. Nuclear signaling pathways for polypeptide ligands and their membrane receptors? *FASEB J* **8:**841–847, 1994.