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JAKs, STATs and Src kinases in hematopoiesis

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Hematopoiesis is the cumulative result of intricately regulated signal transduction cascades that are mediated by cytokines and their cognate receptors. Proper culmination of these diverse signaling pathways forms the basis for an orderly generation of different cell types and aberrations in these pathways is an underlying cause for diseases such as leukemias and other myeloproliferative and lymphoproliferative disorders. Over the past decade, downstream signal transduction events initiated upon cytokine/growth factor stimulation have been a major focus of basic and applied biomedical research. As a result, several key concepts have emerged allowing a better understanding of the complex signaling processes. A group of transcription factors, termed signal transducers and activators of transcription (STATs) appear to orchestrate the downstream events propagated by cytokine/growth factor interactions with their cognate receptors. Similarly, cytoplasmic Janus protein tyrosine kinases (JAKs) and Src family of kinases seem to play a critical role in diverse signal transduction pathways that govern cellular survival, proliferation, differentiation and apoptosis. Accumulating evidence suggests that STAT protein activation may be mediated by members of both JAK and Src family members following cytokine/growth factor stimulation. In addition, JAK kinases appear to be essential for the phosphorylation of the cytokine receptors which results in the creation of docking sites on the receptors for binding of SH2-containing proteins such as STATs, Src-kinases and other signaling intermediates. Cell and tissue-specificity of cytokine action appears to be determined by the nature of signal transduction pathways activated by cytokine/receptor interactions. The integration of these diverse signaling cues from active JAK kinases, members of the Src-family kinases and STAT proteins, leads to cell proliferation, cell survival and differentiation, the end-point of the cytokine/growth factor stimulus.
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Hematopoiesis, the process of generation of blood cells, is a constant continuum in development since most mature blood cells are short-lived and must be replaced throughout adult life. Blood cells originate from a self-renewing population of multi-potential hematopoietic stem cells that become committed to differentiate along the erythroid, megakaryocytic, granulocytic, monocytic and lymphocytic lineages. It has been largely acknowledged that this process is achieved through an intricate network of complex but finely tuned regulatory pathways (Paulson and Bernstein, 1995; Zon, 1995; Cantor and Orkin, 2001). However, the precise mechanisms involved in achieving the coordinate production of different types of blood cells as well as regulation of critical events of differentiation commitment and cellular maturation are still vague. Over the past several years, it has become increasingly clear that hematopoietic cell proliferation and differentiation is controlled by a family of soluble polypeptides referred to as cytokines which include the interleukins, interferons, colony-stimulating factors and erythropoietin (Metcalf, 1989; Kishimoto *et al*., 1994).

Advances in hematopoietic cell culture have allowed the expansion of certain populations of hematopoietic cells in the presence of cytokines and the availability of these cell lines has allowed *in vitro* investigations on the mechanisms by which these cytokines direct signal transduction in the hematopoietic cell. Some of these cell lines display dramatic morphological changes, which occur during maturation of hematopoietic cells in response to colony stimulating factors. A commitment process can be observed in established myeloid leukemia cell lines. During cell division these cells have the option of self-renewal, that is replication without commitment, or irreversible commitment to the formation of differentiated progeny. Self renewal can be suppressed and differentiation commitment can be induced in such cell lines by more than one cytokine: for example, leukemia inhibitory factor (LIF) or interleukin-6 induce differentiation in M1 cells (Lord *et al*., 1991; Selvakumaran *et al*., 1992).

Similarly, the interleukin-3 dependent murine myeloid cell line 32Dcl3 can be induced to undergo differentiation to either granulocytes or macrophages depending upon the stimulus provided. Incubation of the 32Dcl3 cells with the granulocyte colony stimulating factor (G-CSF), results in the differentiation of

32Dcl3 cells to neutrophilic granulocytes and exposure of these cells to granulocyte-macrophage colony stimulating factor (GM-CSF) results in differentiation to macrophages (Valtieri *et al*., 1987; Kreider *et al*., 1990; Rovera *et al*., 1987; Migliaccio *et al*., 1989). Utilization of these cell lines as model systems to investigate the signal transduction pathways initiated by these cytokines and CSFs has resulted in a better understanding of the players involved in hematopoietic cell development. Moreover, molecular cloning of the cDNA's and purification of several of these cytokines has further aided in a more precise definition of their structural features and mechanism of action. This information has opened the door for testing their clinical value in immunological, hematological and malignant disease.

Cytokines and their receptors

Over the past several years dedicated work from several groups has established that hematopoietic cell growth and differentiation is mediated by a group of soluble factors known as cytokines (Kishimoto *et al*., 1994; Hunter, 1993; Stahl and Yancopoulos, 1993; Taniguchi, 1995). Cytokines bind to their cognate receptors and mediate intracellular signal transduction events that result in the modulation of gene expression. Even though there has been considerable advance in our understanding of the processes that determine the physiology of cytokine action, many issues still remain uncertain. There are several reasons for this lack of a complete understanding of cytokine biology. This complexity of cytokine action stems from their functional pleiotropy i.e. a particular cytokine can exhibit a wide variety of biological functions on various tissues and cells. Interleukin-6 (IL-6) is a typical example of such a multifunctional cytokine. IL-6 exerts its effects not only on the B-cell maturation process but also on T cells, hepatocytes, hematopoietic progenitor cells and neuronal cells (Kishimoto, 1989; Kishimoto *et al*., 1992; Akira *et al*., 1993). To add to this complexity of functional pleiotropy, cytokines also function in a redundant manner and several different cytokines can exert similar and overlapping effects on the same cell type. For example, in addition to IL-6, other cytokines such as IL-2, IL-4, IL-5 and interferon-γ can induce antibody production in B-cells (Kishimoto, 1989). Also, two pleiotropic cytokines, IL-6 and leukemia inhibitory factor (LIF) can induce differentiation of the murine myeloid cell line M1 into macrophages (Selvakumaran *et al*., 1992). It is now clear that such a complexity of cytokine action is the primary reason behind the multi-faceted roles played by cytokines. Therefore, the functional pleiotropy and redundancy is now regarded to be a reflection of similarities or differences in the nature of signal transduction pathways activated by cytokines in the context of a given cell type.

Structurally, most cytokine receptors consist of a multi-subunit protein complex: a unique and specific ligand binding subunit, and a signal transducing subunit, which may be structurally similar to other members of the cytokine receptor superfamily (Kishimoto *et al*., 1994; Hunter, 1993; Stahl and Yancopoulos, 1993; Taniguchi, 1995). The signal transducing subunit not only receives signals initiated upon cytokine binding but it is also responsible for propagation of the signals to downstream target proteins. Therefore, the signal transducing subunit recruits cytoplasmic proteins, which results in changes in protein–protein interactions as well as changes in the phosphorylation status of other proteins. Cytokine receptors share a typical structure consisting of about 210 amino acids in their extracellular domain. These proteins have conserved motifs containing cysteine (C) and tryptophan (W) residues in their N-terminal portion. A typical sequence of tryptophan-serine-X-tryptophan-serine (W-S-X-W-S), where X refers to any non-conserved amino acid residue, is present in the C-terminus (Miyajima *et al*., 1992). Structurally, this region is also composed of two fibronectin type III modules. A hinge region connects these two modules, each of which comprises of approximately 100 amino acids. The hinge region, which also houses the W-S-X-W-S motif, is predicted to function as a ligand interaction site. Site-directed mutational analysis within the hinge region, particularly the W-S-X-W-S motif abolishes or greatly reduces the ligand binding capacity of the receptors (Barry *et al*., 1997).

To allow a better classification tool the cytokine receptor family has been sub-divided based on the characteristic structural motifs in their extracellular domains (Kishimoto *et al*., 1994; Hunter, 1993; Stahl and Yancopoulos, 1993; Taniguchi, 1995). The main sub-types of this large family are (i) the gp130 family, (ii) the IL-2 receptor family, (iii) the growth hormone (GH) family, (iv) the interferon (IFN) family and (v) the gp140 family. A detailed description of the features of each of these families is beyond the scope of this review and this review describes only a few salient features of these protein families. For detailed information on the cytokine receptor families, readers are referred to several expert reviews on the topic (Kishimoto *et al*., 1994; Hunter, 1993; Stahl and Yancopoulos, 1993; Taniguchi, 1995).

The gp130 family

Receptors for IL-6, IL-11, cardiotropin-1 (CT), ciliary neurotrophic factor (CNTF), oncostatin M, leukemia inhibitory factor (LIF), all signal through a common β-chain called gp130. Apart from this common gp130 signal transducing subunit, each of the above mentioned receptor possesses a unique and specific ligand binding α-subunit.

IL-2 receptor family

The IL-2 receptor consists of three subunits, α, β and γ, of which the gamma chain (IL-2Rγ chain) is shared by receptors for the IL-4, IL-7, IL-9 and IL-15. Each of

these receptors, like the IL-2 receptor, consist of three subunits, termed α, β and γ. Of these, the α subunit acts as the ligand binding subunit, while the β and γ subunits function as signal transducing subunits. The IL-2Rγ chain associates with JAK3 and mutations in both IL-2Rγ chain and JAK3 results in a non-functional receptor, which abrogates signaling via IL-2, IL-7, IL-9 and IL-15 (Russell *et al*., 1994, 1995).

The growth hormone (GH) family

This family includes receptors for growth hormone (GH), prolactin, erythropoietin, GCSF and thrombopoietin. These receptors consist of a single subunit that forms homodimers upon ligand binding. While being structurally similar, they do not cross react with ligands of each other and transduce signals which are specific for a cytokine.

The interferon (IFN) family

This family comprises of the IFN alpha receptor (IFNAR), the IFN gamma receptor (IFNGR) and the IL-10 receptor. The IFN gamma receptor also has two chains, IFNGR1 and IFNGR2. The IL-10 receptor comprises of two chains, IL-10R1 and IL-10R2. In the case of these receptors, both subunits function as signal transducing components of the receptor and bind to downstream signaling proteins such as JAKS and STATs.

The gp140 family

This family includes the receptors for cytokines, IL-3, IL-5 and GM-CSF. The IL-3 and GM-CSF genes located on the 5q region of chromosome 5 has been associated with hematopoietic malignancies, underlining the importance of these cytokines in hematopoietic signaling (Blalock *et al*., 1999). The receptors for these cytokines, have unique ligand binding-alpha chains and share the common beta (gp140) signal transducing subunit (Itoh *et al*., 1990; Murata *et al*., 1992; Gearing *et al*., 1989; Hayashida *et al*., 1990). Comprehensive reviews on the structure-function analysis of the gp140 receptor proteins, IL-3R, IL-5R and GM-CSFR, have been recently published (Bagley *et al*., 1997; de Groot *et al*., 1998; Woodcock *et al*., 1999, Reddy *et al*., 2000).

Signal transduction pathways activated by cytokines

This review will address the complex interaction between the apparently divergent but inter-dependent signal transduction pathways using the interleukin-3 receptor model system. IL-3 receptor (IL-3R) which is a member of the gp140 family of cytokine receptors that includes the GM-CSF and IL-5 receptors recruits multiple signal transduction cascades that are represented by members of distinct protein families (Figure 1). The concerted action of members of these protein families: (i) tyrosine or serine/threonine kinases; (ii) adaptor or docking proteins; (iii) transcription factors and (iv) pro- or anti-apoptotic proteins, culminates in the growth and survival phenotype propagated by these cytokines. We describe in brief the signal transduction intermediates that convey the growth and cell survival responses mediated by IL-3. Finally, we provide evidence that elucidates the complex nature of the integration of these diverse signaling intermediates that conforms to our Src-JAK-STAT model.

Tyrosine kinases and signal transduction

Molecular cloning of cytokine receptors and subsequent structure-function studies has revealed that unlike growth-factor receptors, cytokine receptors lack a cytoplasmic kinase domain. Nevertheless, interaction of a cytokine with its receptor has been found to rapidly induce tyrosine phosphorylation of the receptors and a variety of cellular proteins suggesting that these receptors transmit their signals through cellular tyrosine kinases (Kishimoto *et al*., 1994). During the past decade, a large amount of experimental data has accumulated to indicate that most cytokines transmit their signals via a new family of tyrosine kinases termed JAK kinases (Ihle *et al*., 1995, 1997; Darnell *et al*., 1994; Darnell, 1998; Schindler, 1999; Schindler and Darnell, 1995; Ward *et al*., 2000; Pellegrini and Dusanter-Fourt, 1997; Leonard and O'Shea, 1998; Leonard and Lin, 2000; Heim, 1999).

Conventional protein tyrosine kinases (PTKs) possess catalytic domains ranging from 250 to 300 amino acids, corresponding to about 30 kDa (Hanks *et al*., 1988). The location of the kinase catalytic domain, in most enzymes, lies near the carboxy terminus of the molecule, whereas the amino terminus performs a regulatory role (Figure 2). The catalytic domains of PTKs consist of a characteristic signature of conserved amino acid residues. PTKs can be further grouped as members of either the Src subfamily, Tec subfamily or one of the three different growth factor receptor subfamilies, the EGF-receptor subfamily, the insulin receptor subfamily or the PDGF-receptor subfamily. The protein tyrosine kinases encoded by the c-*abl* and c-*fes*/*f*ps genes are distantly related to the *src* subfamily. At least nine of the protein tyrosine kinases including c-*fgr*, c-*src,* c-*abl* and c-*fes* have been transduced by retroviruses, where they perform a transforming function (Pierce, 1989). Apart from the kinase catalytic domains, PTKs possess certain other characteristic motifs that enable the kinases to interact with diverse signaling intermediates (Figure 2). These domains are represented by the (a) Src-homology 2 (SH2), (b) SH3 domains, (c) the pleckstrin homology domain (PH), (d) a negative regulatory tyrosine in the carboxy terminus and (e) myristylation or palmitoylation (lipid) modification sites at the N-terminus (Cooper and Howell, 1993; Pawson, 1995). The PH and the lipid modification sites are considered to be important for attachment of the kinases to membranes. The PH domain may also facilitate association of other

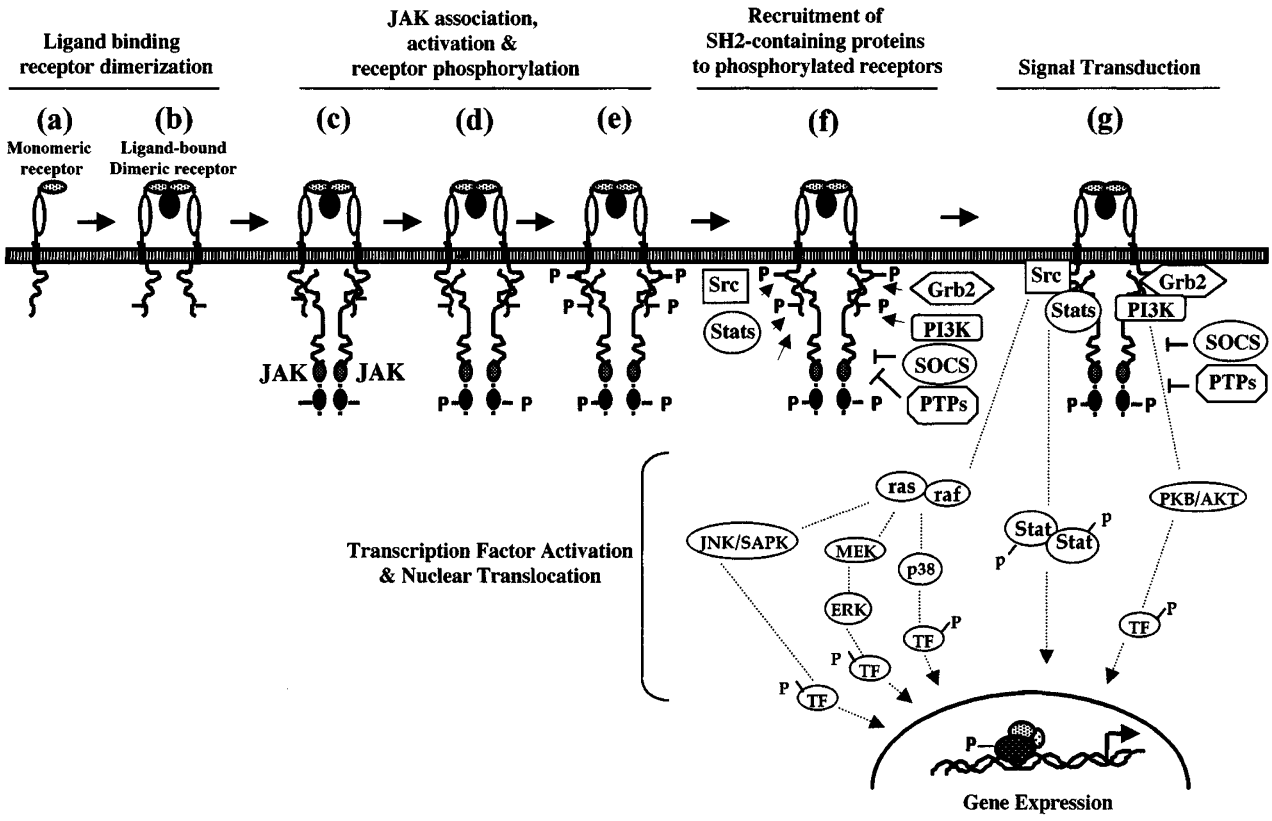

Figure 1 Hierarchy and central components of hematopoietic signal transduction. Hematopoietic signal transduction involves proteins that belong to diverse families. Therefore, an extracellular ligand (cytokine or growth factor) interacts with its receptor (monomeric or dimeric). The ligand-receptor interaction elicits signals that lead to activation of cytoplasmic kinases (JAK kinases, etc) and membrane-localized kinases (Src-kinases, etc). The phosphorylation events elicited by these kinases transmit the signals downstream via other signal mediators such as docking proteins (Grb2, etc), serine/threonine kinases (AKT, PI3K, etc), kinase inhibitors (SOCS/CIS/JAB proteins, PTPs) and transcription factors (STAT proteins). Downstream signal mediators such as members of the MAP/JNK/ERK kinase families further impart signal specificity. The concerted action of all these diverse proteins leads to transmission of the signal to the nucleus to elicit gene expression

signaling proteins to membranes thereby bringing them in close proximity to the kinases. The JAK kinase family, which constitutes a distinct family of tyrosine kinases is a relatively new member of tyrosine kinase gene families and has been the focus of intensive investigation during the past few years. While it was originally thought that this family of kinases is mainly involved in interferon and cytokine mediated signal transduction pathways, recent evidence suggests that these kinases act as mediators of multiple signaling pathways and are essential for the normal function of the mammalian organism. The structure-function analysis of JAK proteins has been addressed elsewhere in detail and is not a focus of this review. However, we will briefly address the important features of JAK proteins as it pertains to their involvement as integral mediators of cytokine signals.

Janus kinases

The unique structure of the JAK kinases clearly distinguishes them from other members of the protein tyrosine kinase family (Figure 2). The most intriguing feature of these proteins is the presence of two domains (JH1 and JH2), with extensive homology to the tyrosine kinase domains. A second interesting feature is the absence of any SH2 or SH3 domains. Instead, these proteins encode a group of well-conserved domains termed as JAK homology (JH1–JH7) domains that follow a non-conserved amino terminus of about 30–50 amino acids. Of the dual kinase domains identified, only the JH1 domain appears to be functional. The JH2 domain, which harbors considerable homology to the tyrosine kinase domains lacks certain critical amino acids required for a functional kinase and does not appear to be associated with a kinase activity. Both the tyrosine kinase domain (JH1) and the pseudo-kinase domain (JH2) are located at the carboxy terminus of the protein. The other conserved blocks of sequences (JH3–JH7) that are characteristic to members of the JAK family, comprise approximately 600 N-terminal amino-acids residues.

The precise functions of the JH3–JH7 domains as well as the pseudokinase domain (JH2) are currently under investigation. The sequences of the JH3–JH7

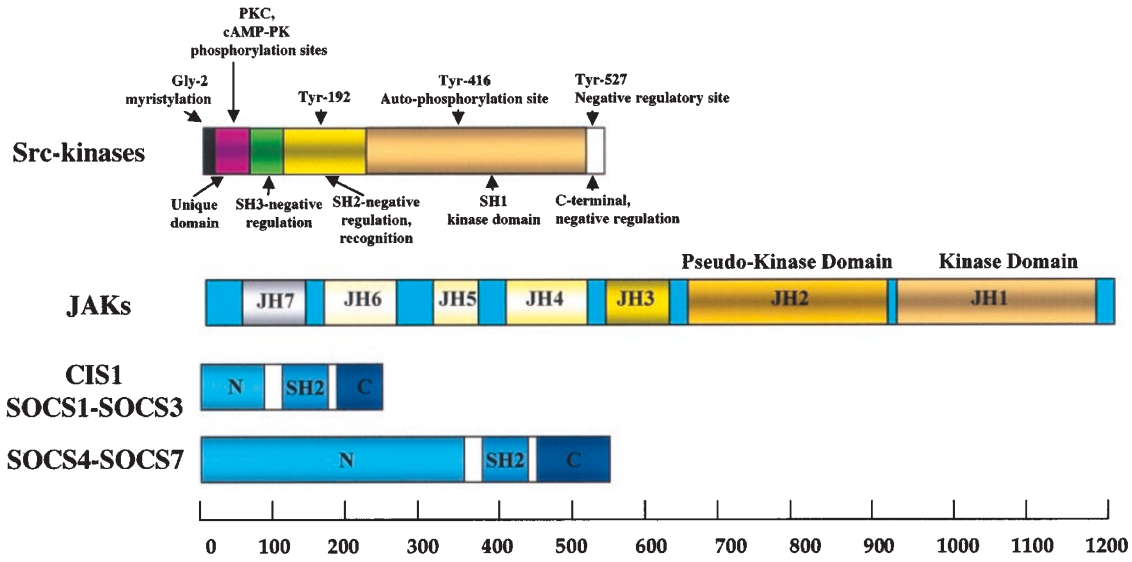

Figure 2 Structural features of Src-kinases, JAKs and SOCS/CIS/JAB protein family proteins. *Src Kinases*: Src kinase family members possess a myristylation signal at the N-terminus that facilitates membrane binding followed by a unique domain that varies in different family members. The SH3, SH2 and SH1 domains that are involved in negative regulation (SH3), negative regulation/recognition/protein–protein association (SH2) and kinase activity (SH1) have conserved amino acid motifs. A conserved auto-phosphorylation site (Tyr-416 in Src) and a negative regulatory tyrosine (Tyr-527 in Src) is also a distinguishing feature of these kinases. *Jak Kinases*: The structural domains featured in the JAK kinase family are referred to as the JAK homology regions (JH1–JH7). JAK kinases, apart from featuring the functional kinase domain (JH1) at the carboxy terminus, also possess a pseudo-kinase domain (JH2). This kinase-like domain in spite of harboring most of the conserved amino acid residues, which are a characteristic of the kinase domain, lacks any observable tyrosine kinase activity. Sequences amino terminal to the kinase and kinase-like domains bear no resemblance to any characterized protein motif. However, there are blocks of homology that are shared among the members of the Janus Kinase family. *SOCS/CIS/JAB proteins*: These proteins are between 250 and 550 amino acids and comprise of conserved N-terminus and C-terminus regions. Interspersed is a conserved SH2 domain that is involved in protein association and kinase inhibition. The conserved SOCS box is present in the C-terminus. The numbers indicate amino acid length

domains bear no resemblance to any characterized protein motif. Considering the variety of interactions and functions performed by the JAK kinase family members it seems plausible that these domains facilitate some key functions like protein–protein interactions, recruitment of substrates, etc. Consistent with this hypothesis, recent studies indicate that the amino terminal regions may be involved in receptor binding (Chen *et al*., 1997; Gauzzi *et al*., 1997). For example, Cacalano *et al*. (1999) recently mapped the region on JAK3 that is obligatory for its functional interaction with the IL-2 receptor. These authors identified a single amino acid substitution (Y100C) in the N-terminus of the JH7 domain of JAK3 in a patient with autosomal severe combined immunodeficiency (SCID). As a result of this mutation, IL-2 responsive signaling was compromised in B-cell lines derived from the patient cells. Furthermore, the authors demonstrated that a region encompassing the JH6 and JH7 domains of JAK3 was sufficient for interaction of the kinase with the proline-rich Box1 region of the IL-2 receptor and was sufficient in reconstituting the IL-2 dependent response. Zhao *et al*. (1995), using a mutational analysis approach demonstrated that truncation of 239 amino terminal residues of JAK2, but not the JH1 and JH2 domains, resulted in abrogation of the interaction of JAK2 with the membrane proximal region of the beta-chain of the GM-CSF receptor. A similar N-terminal truncation of JAK2 also abolished its interaction with the growth hormone receptor (Frank *et al*., 1995). Several other studies that utilized diverse receptor systems have further validated the role of the N-terminus of JAK kinases as modular domains that dictate interactions of the kinases with receptors. Thus, the amino terminal region of JAK1 and JAK2 was required for association with the IFN gamma receptor chains and downstream signaling (Kohlhuber *et al*., 1997).

The kinase like JH2 domain, despite harboring most of the conserved amino acid residues that are a characteristic of the kinase domain, lacks any observable tyrosine kinase activity. It is now clear that this kinase-like domain plays a regulatory role (Luo *et al*., 1997). Saharinen *et al*. (2000) studied the functional significance of the various JAK2 protein domains by mutational analysis. Deletion of the JAK2 pseudo-kinase domain, but not of JH3–JH7 domains, negatively regulated JAK2 catalytic activity as well as STAT-5 activation by JAK2. Furthermore, this study indicated that JAK2 kinase inhibition was mediated by an interaction between the JAK2 kinase and the pseudo-kinase domains. The above results were further substantiated when the authors detected an inhibition of JAK2 single-kinase domain activity upon trans expression of the pseudo-kinase domain, whereas deletion of the pseudo-kinase domain resulted in de-

regulation of JAK2 kinase activity in response to interferon gamma resulting in an increase in STAT activity.

Auto (trans) phosphorylation of conserved tyrosine residues in the JAK kinase activation loop determines the levels of catalytic activation. Such a mode of activation is a conserved feature of tyrosine kinases in general and the tyrosine phosphorylation may allow substrates to gain access to the active site (Duhe and Farrar, 1995). Zhou *et al.* (1997) who engineered point mutations in the activation loop of the JAK3 kinase domain, studied the importance of tyrosine phosphorylation on critical tyrosine residues in modulating JAK kinase catalytic activities. The authors detected multiple auto-phosphorylation sites on JAK3 and mutated two tyrosine residues to phenylalanine (Y980F and/or Y981F). The Y980F mutant exhibited decreased kinase activity, whereas the Y981F mutant exhibited significantly increased kinase activity and the double mutant Y980F/Y981F displayed intermediate kinase activity. Furthermore, the authors also demonstrated that optimal phosphorylation of JAK3 on other phosphorylation sites as well as substrate phosphorylation was dependent on Y980 phosphorylation. Similarly, Gauzzi *et al.* (1996) showed that substitution of two adjacent tyrosine residues in the Tyk2 activation loop with phenylalanine resulted in the abrogation of TYK2 activity in response to IFNα/β.

Receptors are primary substrates of JAK activation

The importance of JAKs in mediating signals from a variety of cytokines/growth factors underscores their importance in signal transduction in general. However, the complexity associated with processing signals from such diverse sources suggests a complicated mechanism of action for the JAK kinases. Although the precise mechanism by which ligand binding results in the activation of JAKs is not known, inferences from results of various studies has allowed the proposition of a model to explain the mechanism of activation of JAK kinases. JAK activation is usually determined by an *in vitro* kinase assay that measures an increase in tyrosine phosphorylation of substrates. JAKs, when expressed in the baculovirus system are enzymatically active and are phosphorylated on tyrosine residues. Their overexpression in mammalian cells also leads to constitutive activation, most probably due to dimerization (Colamonici *et al.*, 1994a,b; Quelle *et al.*, 1995; Duhe and Farrar, 1995; Yamamoto *et al.*, 1994; Wang *et al.*, 1995; Eilers *et al.*, 1996). On the other hand, a JAK kinase in complex with a native un-liganded receptor is in a catalytically inactive latent state. Receptor dimerization/oligomerization due to ligand binding results in the juxtapositioning of the JAKs, which are in the vicinity either through homo- or heterodimeric interactions. This recruitment of the JAK kinases appears to result in their phosphorylation either via autophosphorylation and/or cross phosphorylation by other JAK kinases or other tyrosine kinase family members. This activation is presumed to result in an increased JAK kinase activity. The activated JAKs then phosphorylate receptors on target tyrosine sites. The phosphotyrosine sites on the receptors can then serve as docking sites that allow the binding of other SH2-domain containing signaling molecules such as STATs, Src-kinases, protein phosphatases and other adaptor signaling proteins such as Shc, Grb2 and Cbl.

This model is supported by several studies where investigators have demonstrated the ability of cytokines such as Erythropoietin (EPO) to rapidly induce receptor oligomerization leading to JAK2 activation (Watowich *et al.*, 1994). Furthermore, studies with chimeric receptors, which combine the extracellular domains of the EGF receptor with the cytoplasmic domain of the Epo receptor, also provide evidence in support of a role for receptor dimerization/oligomerization in JAK kinase activation. EGF stimulation of cells expressing the EGF-Epo chimeric receptors, leads to receptor oligomerization of the chimeras followed by activation of the JAK2 kinase and subsequent induction of mitogenesis (Ihle *et al.*, 1995). Similar chimeric receptor based studies have elucidated the importance of oligomerization of the β_c-chain of the receptors for IL-3/IL-5/GM-CSF in signal transduction by their respective cytokines (Eder *et al.*, 1994; Jubinsky *et al.*, 1993). Also, studies with chimeric receptors for IL-2 suggest a similar role for receptor oligomerization as a prerequisite for signal transduction by IL-2 (Watowich *et al.*, 1994). In these cases, as with the EGF-Epo chimeric receptors, the chimeras were functionally capable of induction of mitogenesis supporting the receptor oligomerization concept for JAK kinase activation. Such a model is readily applicable for both single chain receptors such as those for Epo, prolactin, growth hormone and G-CSF as well as multi-chain receptors such as those for IL-3, IL-5 and GM-CSF. Cytokine binding results in the association of the JAKs with one of the subunits. The receptor associated JAK kinase can then either process the signal or subsequent to the receptor oligomerization can recruit other JAKs in the vicinity. Homo or heterodimerization of the JAKs followed by their activation upon phosphorylation finally results in the propagation of the initial signal.

While the importance of receptor oligomerization in the activation of the JAK kinases was being documented, other studies focused on delineating the regions of the receptors that play a critical role in JAK-receptor interactions. These studies clearly demonstrated the importance of membrane proximal domains of the cytokine receptors containing the Box 1 and Box 2 motifs in receptor/JAK interactions. The Box 1 motif comprises of approximately eight proline-rich amino acids, that are required for interaction of several cytokine/growth factor receptors with JAK2. Thus, the Box 1 region of the receptors for prolactin, growth hormone, erythropoietin, IL-6 and related cytokines, that utilize the gp130 signal transducing receptor subunit, is required for association with JAK2 (Witthuhn *et al.*, 1993; Miura *et al.*, 1994; Tanner *et*

al., 1995; VanderKuur *et al*., 1994; Goujon *et al*., 1994; Hackett *et al*., 1995; DaSilva *et al*., 1994). Jiang *et al*. (1996) used mutational analysis to demonstrate that the Box 1 region of cytokine receptors specifies the region of interaction with JAK kinases. IL-2 stimulation leads to the activation of JAK3 but not of JAK2 whereas erythropoietin conveys its signals by recruiting and activating JAK2. These authors created chimeric receptor molecules by switching the Box 1 region of the EpoR with that of the beta chain of the IL-2R. The EpoR-IL-2R fusion receptor was sufficient to induce activation of JAK2 in response to stimulation with IL-2 thereby confirming the role of Box 1 region in determining the specificity of JAK kinase activation. Mutational analysis of several receptors corroborates such a theory. Mutations in the membrane proximal regions of the Epo receptor resulted in the elimination of JAK2 association and activation as well as abrogation of mitogenesis in response to erythropoietin (Witthuhn *et al*., 1993). Similar mutational analysis demonstrated the importance of the membrane proximal domains of the prolactin receptor in association and activation of JAK2 (DaSilva *et al*., 1994). Also, the Box 1 and Box 2 regions of gp130 were shown to be required for association and activation of JAK1 and JAK2 (Narazaki *et al*., 1994). The requirement of membrane proximal motifs in JAK kinase interaction is absolute even in the case of receptors that lack Box 1 motifs. JAK1 and JAK3 constitute integral components of the IL-2R signal transduction apparatus. The IL-2R gamma-c chain does not contain a typical Box 1 motif and yet can activate the JAK1 and JAK3 kinases. It has been determined that a membrane proximal region of the IL-2R encompassing 52 amino acids is sufficient for JAK3 binding, activation of JAK1 and JAK3 and other downstream signaling events such as induction of transcription factors c-*fos* and c-*myc* and cell proliferation (Nelson *et al*., 1996). These studies indicate that aberrations at any step of the aggregation of ligand-receptor/JAK kinase hetero-oligomeric complexes could be sufficient to abolish activation of the kinases and prevent mitogenesis, most probably by eliminating indispensable downstream signaling events.

IL-3/IL-5/GMCSF activation of hematopoietic cells appears to result predominantly in the activation of JAK2, although JAK1 and TYK-2 have also been found to be activated in certain cell systems (Silvennoinen *et al*., 1993a,b; Quelle *et al*., 1994; Lutticken *et al*., 1994; Chaturvedi *et al*., 1997, 1998; Jaster *et al*., 1997). The membrane proximal region of the IL-3R β_c chain is involved in association with the N-terminal region of JAK2. Deletion of this region of the IL-3R β_c chain results in loss of activation of JAK2 (Quelle *et al*., 1994). In addition, the cytoplasmic portion of the α-chains of IL-3, GM-CSF and IL-5 receptors also appear to be involved in the activation of JAK2 kinase (Quelle *et al*., 1994; Cornelis *et al*., 1995; Kouro *et al*., 1996; Matsuguchi *et al*., 1997; Takaki and Takatsu, 1994). Activation of JAK2 leads to phosphorylation of the IL-3R β_c chain on multiple tyrosine residues (Y577, Y612, Y695 and Y750) which in turn serve as docking sites for other signal transducing proteins, the most important of which are the STATs (Durstin *et al*., 1996; Inhorn *et al*., 1995; van Dijk *et al*., 1997; Pazdrak *et al*., 1997; Chin *et al*., 1996; Woodcock *et al*., 1996).

Aberrations in JAK kinase activity, that may lead to derailment of one or more of the above mentioned pathways could disrupt normal cellular responses and result in disease states. Thus, over-activation of JAK kinases has been implicated in tumorigenesis. In contrast, loss of JAK kinase function has been found to result in disease states such as severe-combined immunodeficiency. In summary, optimal JAK kinase activity is a critical determinant of normal transmission of cytokine and growth factor signals.

STAT signaling pathways

Over the past few years, researchers have deciphered a signaling pathway, which initiates within the cytoplasm but quickly translocates to the nucleus to activate transcription of target genes. This novel signaling pathway features a group of transcription factors named as STATs or Signal transducers and Activators of Transcription (Schindler and Darnell, 1995; Darnell, 1997; Bromberg and Chen, 2001). These transcription factors were originally described by Darnell and his co-workers (Darnell *et al*., 1994; Darnell, 1997) as ligand-induced transcription factors in interferon-treated cells. Subsequent studies by a number of groups showed that STATs play a critical role in signal transduction pathways associated with several cytokines and neurokines including the interleukins, the interferons, erythropoietin, prolactin, growth hormone, oncostatin M (OSM), and ciliary neurotrophic factor (CNTF) (Darnell *et al*., 1994; Darnell, 1997). To-date, six mammalian genes that code for different STATs have been identified, all of which encode for proteins of 750–850 amino acids long and are characterized by the presence of a DNA-binding domain followed by putative SH3 and SH2 domains (Darnell, 1997). In addition, alternative splicing or post-translational proteolytic cleavage reactions appear to generate additional forms for STAT-1, -3 and -5, bringing the total number of STATs currently described in literature to eight (Figure 3). Thus, two isoforms of STAT-1 have been described, which have been termed as STAT-1 α or p91 and STAT-1 β or p84. These two proteins were originally discovered by Darnell's group in association with STAT-2 and a fourth protein termed p48, which constituted the multicomponent transcription factor ISGF3. STAT-3 also exists in two forms termed STAT-3A and STAT-3B with different transcriptional activation functions (Schaefer *et al*., 1995). STAT-5 exists in two isoforms, termed as STAT-5A and STAT-5B, which are encoded by two separate genes, which are tandemly linked on human chromosome 17 and mouse chromosome 11 (Lin *et al*., 1996; Copeland *et al*., 1995). The two proteins exhibit extensive sequence homology and differ from each

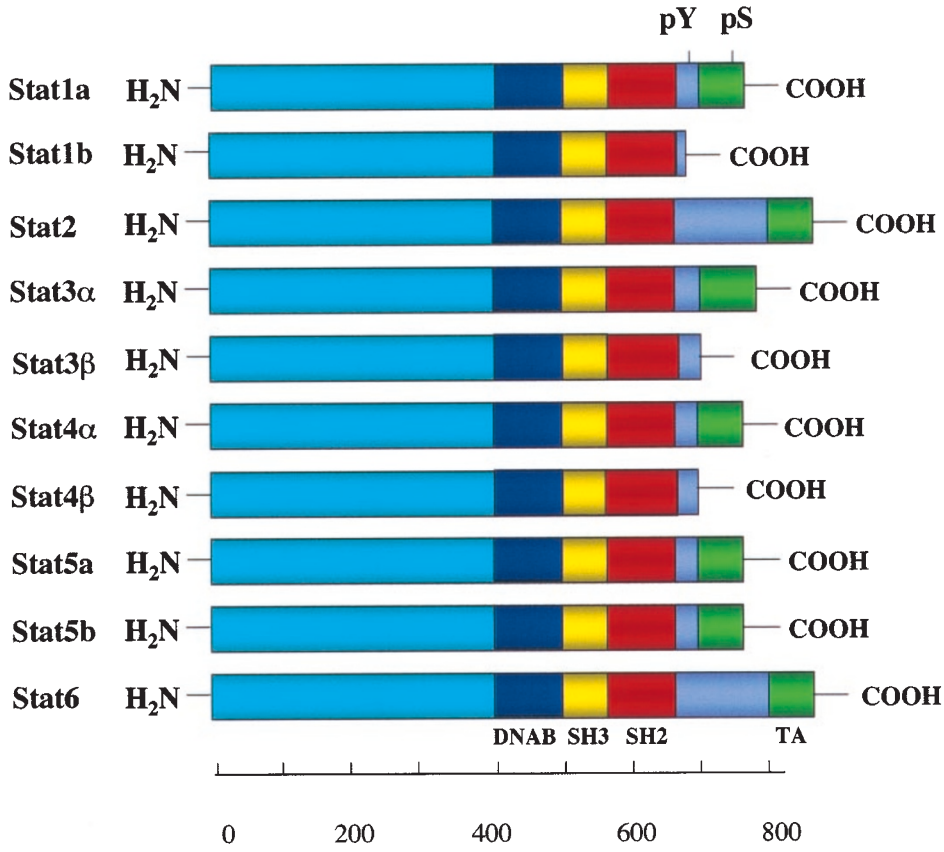

Figure 3 STAT protein structure. Cytokines signal via members of the STAT family of proteins. The various STAT proteins, many of which are derived as a result of alternative splicing events (e.g.: Stat 1, Stat 3, Stat 4 and Stat 5) comprise of approximately 800 amino acid residues. Sequence analysis indicates that the STAT proteins harbor conserved motifs, such as the DNA binding region (DNAB), SH2 and putative SH3 domains that allow DNA-protein and protein–protein interactions. The transactivation (TA) domain and the highly conserved phosphorylatable tyrosine (pY) and serine (pS) residues that are essential for optimal STAT activation reside at the carboxy terminus of the proteins

other mainly in the amino and carboxy terminal domains, with the transactivation domain showing most divergence. Both of these genes seem to play a critical role in IL3/IL-5/GMCSF-induced proliferation of hematopoietic cells as well as prolactin-induced proliferation of mammary epithelial cells (Takaki *et al*., 1994; Quelle *et al*., 1994; Cornelis *et al*., 1995; Matsuguchi *et al*., 1997; Itoh *et al*., 1996).

Like most transcription factors, STATs exhibit a modular structure with five well defined domains, which include the N-terminal conserved domain, the DNA-binding domain, a putative SH3-like domain, a SH2 domain and C-terminal transactivation domain (Figure 3). The amino terminal region of STATs is well conserved and appears to be critical for STAT function as small deletions in this region were found to eliminate the ability of STATs to be phosphorylated. Vinkemeier *et al*. (1998) have resolved the crystal structure of an NH2-terminal conserved domain (N-domain) comprising the first 123 residues of STAT-4 at 1.45 angstroms. Their studies suggest that the domain consists of eight helices that are assembled into a hook-like structure. Several studies have implicated the N-domain in various protein–protein interactions affecting transcription and it has been suggested that this domain enables dimerized STAT molecules to polymerize and to bind DNA cooperatively. The structure shows that STAT N-domains can interact through an extensive interface formed by polar interactions across one face of the hook. In addition, mutagenesis of an invariant tryptophan residue at the heart of this interface resulted in abrogation of cooperative DNA binding by the full-length protein *in vitro* and reduced the transcriptional response after cytokine stimulation *in vivo*.

This region is followed by the DNA binding domain that is usually located between amino acids 400 and 500 (Horvath *et al*., 1995). This region is highly conserved amongst the STATs, and all STATs with the exception of STAT-2 differentially bind more than 10 related GAS sequences that are characterized by the consensus sequence motif, TTNCNNNAA (Horvath *et al*., 1995; Xu *et al*., 1996). Adjacent to the DNA binding domain lies the putative SH3 domain. This domain appears to be least conserved and it is at present unclear as to whether it truly functions as a SH3 domain, since the critical amino acid residues involved in binding to PXXP motifs do not appear to

be well conserved. Adjacent to this putative SH3 domain lies the SH2 domain, which is highly conserved amongst the STATs and appears to play a very important role in STAT signaling. Thus, it is critical for the recruitment of STATs to the activated receptor complexes and is required for the interaction with JAK and Src kinases. In addition, it is required for STAT homo and hetero dimerization, which in turn appears to be critical for nuclear localization and DNA binding activities. It is also possible that this domain participates in other protein–protein interactions that have not yet been fully deciphered.

All members of the STAT family are tyrosine phosphorylated in response to cytokine stimulation at a conserved carboxy-terminal tyrosine, Y694, in the case of STAT-5. To determine structural features important for STAT signaling, Barber *et al.* (2001) generated an activation-specific STAT-5 antibody using a phosphopeptide containing amino acids 687–698 of STAT-5 as antigen that specifically recognizes tyrosine-phosphorylated STAT-5 but not nonphosphorylated STAT-5. In immunoprecipitation reactions activation-specific STAT-5 antibodies selectively immunoprecipitated the tyrosine phosphorylated EPO receptor (EPO-R) in addition to STAT-5 under native and denaturing conditions. Based on these studies, the authors proposed that the activation-specific STAT-5 antibody recognizes the two substrates to which the STAT-5 SH2 domain interacts, namely, the tyrosine-phosphorylated EPO-R and STAT-5 itself. Furthermore, using a series of EPO-R tyrosine mutants expressed in Ba/F3 cells, they have shown that the activation-specific STAT-5 antibody immunoprecipitates an EPO-R containing only two tyrosines at positions 343 and 401, confirming the importance of these tyrosines in STAT-5 recruitment.

In addition, Kovarik *et al.* (2001) have demonstrated that the specificity of signaling by STAT-1 depends on SH2 and C-terminal domains that regulate Ser727 phosphorylation and thereby differentially affecting specific target gene expression. It is known that complete activation of STAT-1 requires phosphorylation at both Y701 and a conserved PMS(727)P sequence. Interestingly, S727 phosphorylation of STAT-1 in interferon-gamma (IFN-gamma)-treated mouse fibroblasts occurred without a need for p38 mitogen-activated protein kinase (MAPK), extracellular signal-regulated kinases 1 and 2 or c-Jun kinases, and required both an intact SH2 domain and phosphorylation of Y701. In contrast, UV irradiation-induced STAT-1 phosphorylation on S727 required p38MAPK, but no SH2 domain-phosphotyrosine interactions. Mutation of S727 differentially affected IFN-gamma target genes, at the level of both basal and induced expression. Furthermore, the PMS(727)P motif of STAT-3 was phosphorylated by stimuli and signaling pathways different from those for STAT-1 S727 and transfer of the STAT-3 C-terminus to STAT-1 changed the stimulus and pathway specificity of STAT-1 S727 phosphorylation to that of STAT-3. This very important series of experiments suggested that STAT C-termini contribute to the specificity of cellular responses by linking individual STATs to different serine kinase pathways and through an intrinsically different requirement for serine phosphorylation at different target gene promoters.

Immediately downstream of the SH2 domain, around position 700, all STATs contain a tyrosine residue, which plays a critical role in STAT activation. Phosphorylation of this tyrosine residue has been found to be essential for the activation and dimerization of STATs. Phosphorylation of this tyrosine appears to be achieved by growth factor receptors as well as JAK and Src kinases, depending on the nature of the cell type and the ligand/receptor interactions. The C-terminal domain of STATs is required for transcriptional transactivation. Phosphorylation of a single serine residue in the TA domains of STAT-1, STAT-3, STAT-4 and STAT-5, can enhance the transcriptional activity. Differentially spliced STATs such as STAT-1β, lacking the C-terminal TA domain can still bind DNA but do not activate transcription.

Chen *et al.* (1998) have resolved the crystal structure of the DNA complex of a STAT-1 homodimer at 2.9 A resolution. Their results indicate that STAT-1 utilizes a DNA-binding domain with an immunoglobulin fold, similar to that of NFkappaB and the p53 tumor suppressor protein. The STAT-1 dimer forms a contiguous C-shaped clamp around DNA that is stabilized by reciprocal and highly specific interactions between the SH2 domain of one monomer and the C-terminal segment, phosphorylated on tyrosine, of the other. In addition, the structure also indicates that the phosphotyrosine-binding site of the SH2 domain in each monomer is coupled structurally to the DNA-binding domain, suggesting a potential role for the SH2- phosphotyrosine interaction in the stabilization of DNA promoter sites.

STATs, which are normally localized in the cytoplasm, are activated when phosphorylated on the tyrosine located around residue 700, which facilitates their dimerization and translocation to the nucleus (Darnell *et al.*, 1994; Schindler and Darnell, 1995). The phosphorylation of STATs is known to occur immediately after the binding of growth factors or interferons to their receptors. Studies with interferon (IFN) receptor signaling have revealed that JAK family kinases are involved in IFN-specific gene expression in cooperation with STATs. Studies with mutant cell lines, which were unable to respond to interferons, described earlier, clearly established a critical role for JAKs in the interferon signaling pathways.

Since the cytokine and interferon receptor-ligand interactions were found to result in the activation of JAK kinases, which often exist in association with cytokine receptors, and this activation was obligatory for the activation of STATs, it was proposed that STATs might be substrates for JAK kinases. This notion was further supported by studies, which showed *in vitro* phosphorylation of purified STAT-5 by JAK2 (Flores-Morales *et al.*, 1998). In this study, the investigators expressed STAT-5 in Sf9 insect cells using

the baculovirus expression system and showed that this recombinant protein can be phosphorylated by JAK2 that confers DNA binding property to STAT-5. In addition, these investigators showed STAT-5 in its non-phosphorylated form was able to form a stable complex with activated JAK2, while non-activated JAK2 and phosphorylated STAT-5 were unable to participate in complex formation. A second line of evidence, which suggested that JAKs might be activators of STATs, came from the use of dominant negative mutants of JAK2. Over-expression of dominant negative JAK1 and TYK2 mutants in human cells suppressed transcriptional activation of a luciferase reporter gene downstream from an ISRE. In addition, these dominant negative mutants were found to inhibit STAT-1 and STAT-2 phosphorylation (Krishnan *et al*., 1997).

However, the activated JAK kinases do not seem to exhibit specificity for a particular STAT as different receptors activate a common STAT, even though they activate distinctively different JAK kinases (Kohlhuber *et al*., 1997; Darnell, 1997). In addition chimeric receptor molecules with different JAK binding sites but containing the same STAT-binding site were found to activate the same STAT (Kotenko *et al*., 1996; Kohlhuber *et al*., 1997). Thus, the specificity for STAT phosphorylation appears to be determined by the docking sites for STATs that are present in the receptor molecules and not JAK kinases.

The two isoforms of STAT-5, STAT-5a and STAT-5b as well as STAT-6 are activated by receptors for IL-3, GM-CSF and IL-5 (Quelle *et al*., 1995; Azam *et al*., 1995). IL-3 stimulation leads to activation of JAK2 and phosphorylation and activation of STAT-5a (via tyrosine phosphorylation of Y694) and STAT-5b (via tyrosine phosphorylation of Y699). Mutational studies with the IL-3, GM-CSF and IL-5 receptors have shown that JAK2 is essential for STAT activation (Kouro *et al*., 1996; Matsuguchi *et al*., 1997; Caldenhoven *et al*., 1995; Mui *et al*., 1995a,b; Smith *et al*., 1997). However, a C-terminal mutant of the β_c chain has been described that can activate JAK2 but is unable to activate STAT-5 indicating that activation of JAK2 alone is not sufficient for STAT-5 activation (Smith *et al*., 1997).

To investigate the role of signal transducer and activator of transcription (STAT) proteins in granulocyte colony-stimulating factor (G-CSF)-regulated biological responses, McLemore *et al*. (2001) generated transgenic mice with a targeted mutation of their G-CSF receptor (termed d715F) that abolishes G-CSF-dependent STAT-3 activation and also attenuates STAT-5 activation. The resultant homozygous mutant mice are severely neutropenic with an accumulation of immature myeloid precursors in their bone marrow and it was determined that G-CSF-induced proliferation and granulocytic differentiation of hematopoietic progenitors was severely impaired. Also, expression of a constitutively active form of STAT-3 in d715F progenitors nearly completely rescued these defects whereas expression of a dominant-negative form of STAT-3 in wild-type progenitors resulted in impaired G-CSF-induced proliferation and differentiation. These results argue that STAT-3 activation by the G-CSFR is critical for the transduction of normal proliferative signals and may also contribute to differentiation signals.

The JAK-Src-STAT model

A role for Src kinases in STAT activation was first suggested by studies aimed at investigating the molecular mechanisms associated with Src-mediated transformation of fibroblasts and hematopoietic cell lines. Thus Src-transformed NIH3T3 cells were found to express a tyrosine phosphorylated form of STAT-3 in a constitutive manner (Yu *et al*., 1995; Cao *et al*., 1996). In addition, results from Cao *et al*. (1996) showed that v-Src could bind to STAT-3 and phosphorylate this protein *in vitro*. Transfection of an expression vector coding for a dominant negative mutant of STAT-3 into v-Src-transformed NIH3T3 cells resulted in a block to v-Src-mediated transformation of cells, further enforcing the notion that STAT-3 signaling plays a critical role in this transformation process. Recently, Okutani *et al*. (2001) explored the function of Src downstream of the EPOR-initiated signaling. Reduction of Src diminished tyrosine phosphorylation of STAT-5 in K562 cells regardless of EPO treatment. In addition, the tyrosine phosphorylation level of STAT-5 induced by EPO in F-36P cells was reduced in the presence of Src inhibitors. Furthermore, the expression of dominant negative Src in F-36P cells reduced the tyrosine phosphorylation of STAT-5 and co-expression of Src and STAT-5 in COS7 cells resulted in tyrosine phosphorylation of STAT-5 where the tyrosine residue 694 (Tyr 694) of STAT-5A was identified as the major phosphorylation site by Src. Furthermore, *in vitro* kinase assays revealed that GST-STAT-5 fusion protein with the conserved C-terminal, but not the C-terminal-truncated mutant which lacks Tyr 694, was tyrosine-phosphorylated by Src. These studies indicate that Src can directly tyrosine-phosphorylate the activation site of STAT-5 (Tyr 694 in STAT-5A).

STAT protein activation has been observed in diverse cell types. Also, constitutive STAT activation has been detected in a wide variety of human primary tumor specimens and tumor cell lines including blood malignancies, head and neck cancer, and breast cancer. Garcia *et al*. (2001) have recently demonstrated that Src and JAK family tyrosine kinases cooperate to mediate constitutive Stat-3 activation in the absence of EGF stimulation in human breast cancer cell lines. These studies show evidence that inhibition of Src or JAKs results in dose-dependent suppression of Stat-3 DNA-binding activity, which is accompanied by growth inhibition and induction of programmed cell death and transfection of a dominant-negative form of Stat-3 leads to growth inhibition involving apoptosis of breast cancer cells. These results indicate that Src and

JAK tyrosine kinases transduce signals through Stat-3 protein that contribute to the growth and survival of human breast cancer cells in culture and potentially *in vivo*.

In our studies aimed at delineating the molecular mechanisms associated with v-Src-mediated transformation of hematopoietic cells, we used 32Dcl3 cells that are derived from normal mouse bone marrow and require IL-3 for proliferation and survival. These cells, when cultured in the presence of G-CSF were found to terminally differentiate into mature granulocytes in a period of 10–12 days (Valtieri *et al*., 1987). When these cells were grown in a medium lacking IL-3 or G-CSF, they were found to undergo apoptosis in a period of 24–48 h. This cell line, when transformed with expression vectors containing v-*abl* or *bcr-abl* or v-*src* was found to grow indefinitely in the absence of IL-3 and to form tumors in nude mouse assays (Kruger and Anderson, 1991, Laneuville *et al*., 1991; Rovera *et al*., 1987). In addition, expression of either v-*src*, v-*abl* or *bcr-abl* was found to block the ability of 32Dcl3 cells to undergo GCSF-induced differentiation (Rovera *et al*., 1987). Interestingly, several other tyrosine kinases closely related to v-*src* failed to induce such cytokine-independence of these cells suggesting a very specific target for v-*src* in these cells. Thus, when v-*fgr* or an activated form of c-*fgr* which lacks the C-terminal 12 amino acids (c-*fgr*D) were expressed in this cell line, they failed to render these cells IL-3 independent for growth (Chaturvedi *et al*., 1998).

To understand the possible mechanisms underlying the IL-3 independent growth of v-*src*-transformed 32Dcl3 cells, we examined the phosphorylation status of JAK1, JAK2 and JAK3 kinases in the v-*src* and v-*fgr*-transformed cells. These studies showed that none of the JAK kinases were phosphorylated by v-Src or v-Fgr. However, v-*src*-transformed cells were found to express constitutively phosphorylated forms of STAT-1, -3 and -5, which exhibited appropriate DNA binding activities. Our results also showed that STAT-3 co-immunoprecipitates with v-Src suggesting that the activation of STAT-3 occurs due to direct association with v-Src (Chaturvedi *et al*., 1998). Since hematopoietic cells express high levels of c-Src and since Src-kinases associate with IL-3 receptor following its activation by IL-3, this observation suggested that c-Src might play a role in the activation of STAT-3 in normal hematopoietic cells following IL-3 stimulation. This was further confirmed by our studies where we examined the phosphorylation status of JAKs and STAT-3 in 32Dcl3 cells following IL-3 stimulation. These studies showed that IL-3 stimulation induces rapid tyrosine phosphorylation of JAK1 and JAK2 in 32Dcl3 cells. In addition, our results show that interaction of IL-3 with its receptor leads to the phosphorylation and nuclear translocation of STAT-1, STAT-3 and STAT-5. In addition, this ligand/receptor interaction leads to the activation of c-Src kinase activity, which in turn facilitates the binding of c-Src to STAT-3. This association leads to the phosphorylation of STAT-3, allowing STAT-3 to translocate to the nucleus. Expression of a dominant negative mutant of *src* (AMSrc) in these cells results in a block to IL-3 mediated phosphorylation of STAT-3, and its ability to bind to DNA (Chaturvedi *et al*., 1998). On the other hand, expression of a dominant negative mutant of JAK2 (JAK2KE) had no effect on IL-3-mediated activation of STAT-3. Our results also show that AMSrc does not affect the phosphorylation of JAK2, suggesting that two independent pathways mediate JAK and STAT-3 phosphorylation events. These results suggest that Src family kinases mediate the phosphorylation of STAT-3 mediated by IL3/receptor interactions and play a critical role in signal transduction pathways associated with myeloid cell proliferation. These results suggest that activation of STAT-3 following cytokine stimulation of certain hematopoietic cells might follow a very different pathway from that seen with STAT-5. These results taken together indicate that while one or both isoforms of STAT-5 interact directly with JAK2, which in turn mediates their phosphorylation, STAT-3 activation might require its interaction with c-Src, which in turn mediates its phosphorylation (Chaturvedi *et al*., 1998). Based on these results, we proposed the JAK-SRC-STAT model, where JAK kinases may be more crucial to phosphorylation of the cytokine/growth factor receptors (Figure 4). Moreover, JAK-mediated phosphorylation may create docking sites on the receptors for binding of SH2-containing proteins such as STATs, Src-kinases and other signaling intermediates (Reddy *et al*., 2000). JAKs or Src-kinases, depending on the nature of STAT that is being activated then induce tyrosine phosphorylation and activation of STAT proteins.

The notion that different STATs might be phosphorylated by different tyrosine kinases under different conditions is suggested by few other observations. Like v-Src, v-Abl and BCR-ABL have been shown to transform hematopoietic cells and render them cytokine-independent for growth (Pierce *et al*., 1985; Cook *et al*., 1985; Mathey-Prevot *et al*., 1986). Thus, v-Abl encoded by the Abelson murine leukemia virus was found to transform both B-cells and myeloid cells resulting in cytokine-independent growth of these cells (Pierce *et al*., 1985; Cook *et al*., 1985; Mathey-Prevot *et al*., 1986). Examination of the molecular mechanisms associated with v-Abl mediated transformation show that B-cells transformed by this oncogene exhibit constitutively activated forms of JAK1, JAK3 as well as STAT-1, -3 and -5 (Danial *et al*., 1995, 1998). In addition, activated JAK1 in these cells was found to be associated with the v-Abl protein. Mapping the JAK1 interaction domain in v-Abl showed that a region of the C-terminal domain of v-Abl protein binds to JAK1 and a mutant of v-Abl that lacks this JAK1 binding domain failed to activate JAK1 and STAT proteins. In addition, a dominant negative mutant of JAK1 was found to inhibit STAT activation mediated by v-Abl suggesting that the v-Abl protein activates STATs via activation of JAK1. It is also interesting to note that a C-terminal deletion mutant of IL-3 βc chain, termed βc541 was found to be deficient in its ability to activate

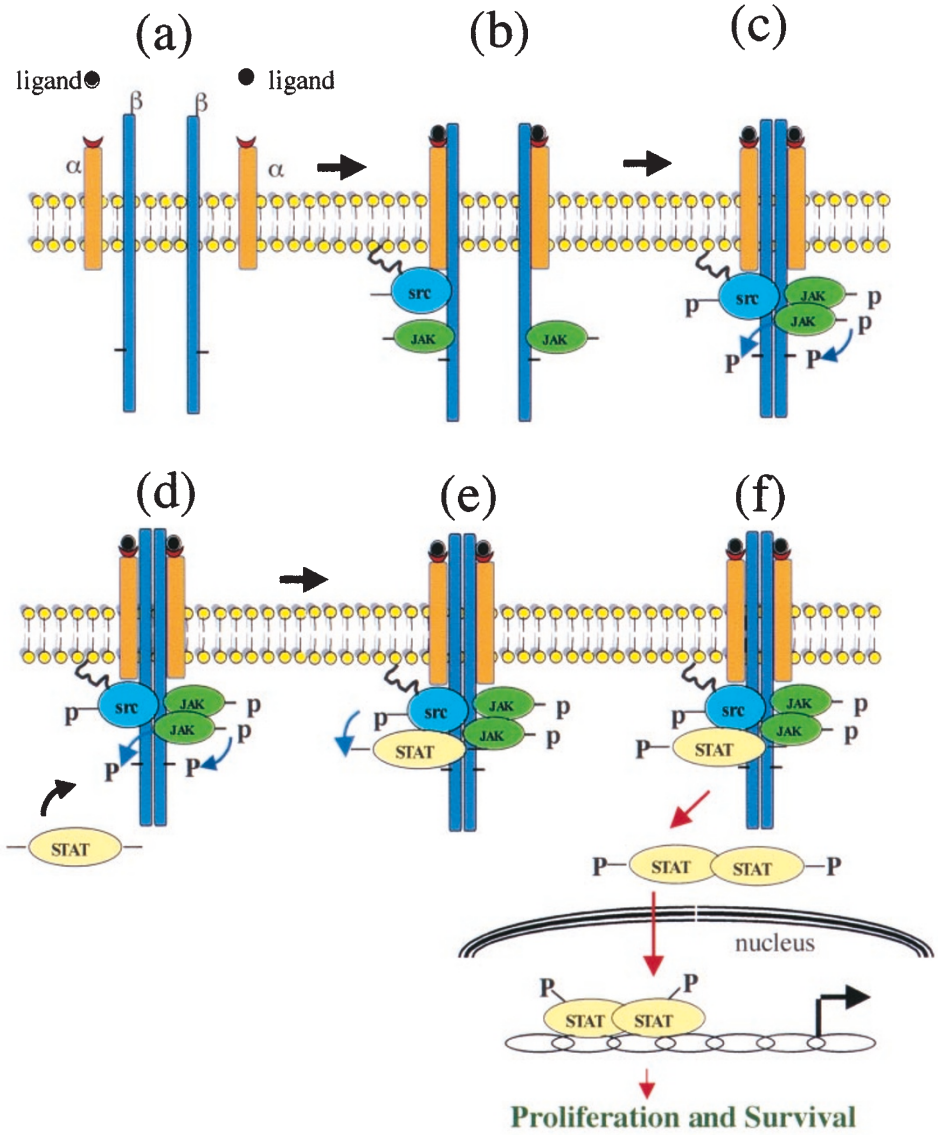

Figure 4 Cytokine receptor-JAK-Src kinase-STAT model of signal transduction. According to this model, (**a**) cytokine or growth factor interactions with their specific receptors lead to the (**b**) activation of JAK kinases and Src kinases resulting from their association with the activated receptor. (**c**) JAK kinases then mediate the phosphorylation of tyrosines on the receptor, which (**d**) serve as docking sites for STATs. (**e**) These STATs are then phosphorylated by Src kinases resulting in their (**f**) dissociation from the receptor, dimerization and translocation to the nucleus where they activate gene transcription

STAT-5, while still being able to activate JAK2 (Smith *et al*., 1997). These observations also imply that while JAK2 activation is a critical step in IL-3/IL-5/GMCSF mediated activation of their cognate receptors, it is by itself not adequate for STAT activation.

In sharp contrast, the BCR-ABL oncogene, a gene closely related to the v-*abl* oncogene was found to constitutively activate STAT-1 and STAT-5 with very little or no activation of JAKs (Carlesso *et al*., 1996; Ilaria and Van Etten, 1996; Frank and Varticovski, 1996; Nieborowska-Skorska *et al*., 1999) Unlike with v-*abl* transformed B-cells, over-expression of dominant negative JAK kinase mutants in these cell lines had no effect on STAT phosphorylation (Ilaria and Van Etten, 1996). Similarly, K562 cells, which are Philadelphia-chromosome positive express constitutively activated STAT-5 and over-expression of a dominant negative STAT-5 could suppress the transformed state of these cells. Activation of STATs by BCR-ABL oncogene was shown to be dependent on the presence of the SH2 and SH3 domains (Nieborowska-Skorska *et al*., 1999), suggesting a Src-like interaction reported by us (Chaturvedi *et al*., 1998). These observations would also provide a molecular basis for the inability of v-Abl to directly activate STATs, since this oncoprotein lacks the SH3 domain that is present in the BCR-ABL oncogene (Reddy *et al*., 1983).

A second line of evidence comes from the study of Kazansky *et al*. (1999) who studied the DNA-binding

and tyrosine phosphorylation of STAT-5A and STAT-5B following prolactin activation or Src activation. Their results show that following prolactin activation, both STAT-5A and STAT-5B were rapidly phosphorylated and translocated to the nucleus. Similar to prolactin activation, *src* activation resulted in tyrosine phosphorylation and DNA binding of both STAT-5A and STAT-5B. Furthermore, overexpression of a dominant negative mutant of JAK2 prevented prolactin-induced tyrosine phosphorylation and nuclear translocation of STAT-5A and STAT-5B. In sharp contrast, over-expression of dominant negative JAK2 had no effect on Src-mediated phosphorylation of STATs. In contrast, there was a modest increase in the levels of Src-mediated phosphorylation of STAT-5 in the presence of dominant JAK2. These studies again suggest that there exist two independent pathways that mediate STAT activation, one that is dependent on JAKs and the other that is dependent on Src kinases. The context of the cytokine and cell type appears to play a critical role in determining as to which pathway is utilized by the cell.

As a follow-up to their prior study, Kazansky and Rosen (2001) have recently described the functional consequences of STAT-5B activation by v-*src*. To this end, they studied the properties of stably transfected NIH3T3 cells containing both an intact and a dominant negative, COOH-terminal-truncated isoform of STAT-5B. According to their studies, STAT-5B enhanced the transforming potential of v-Src as reflected by both an increase in focus formation and growth in soft agar and enhanced v-Src-induced cell cycle progression and cell motility in NIH3T3 cells. Furthermore, the dominant negative, COOH-terminal-truncated isoform of STAT-5B was able to partially suppress v-Src-mediated cell transformation that supports the hypothesis that STAT-5B may enhance oncogene-induced tumorigenesis.

Stat-3 has been shown to have an essential role in cell transformation by the Src oncoprotein. Bowman *et al*. (2001) have recently elucidated the mechanisms by which STAT-signaling pathways contribute to mitogenesis and transformation. They showed that disruption of Stat-3 signaling by using dominant-negative Stat-3beta protein in NIH3T3 fibroblasts suppresses c-Myc expression concomitant with inhibition of v-Src-induced transformation. In addition, ectopic expression of c-Myc is able to partially reverse this inhibition, suggesting that c-Myc is a downstream effector of Stat-3 signaling in v-Src transformation. Consistent with these results they observed that fibroblasts derived from c-*myc* null mutant mice are refractory to transformation by v-*src,* consistent with a requirement for c-Myc protein in v-*src* transformation. Also, in normal NIH3T3 cells, disruption of Stat-3 signaling with dominant-negative Stat-3beta protein inhibits PDGF-induced mitogenesis that can also be reversed by ectopic c-Myc expression. In agreement with this, it was found that inhibition of Src family kinases with the pharmacological agent, SU6656, leads to a block to Stat-3 activation by PDGF. Based on these results, the authors argue that PDGF signals are mediated in a hierarchical order that involves activation of Src kinase that leads to activation of STAT-3 and downstream Myc protein and that these proteins are deregulated in Src transformation.

The above mentioned studies have resulted in concerted investigative efforts aimed at blocking aberrant STAT activation. To identify small molecule inhibitors of Stat-3, Turkson *et al*. (2001) investigated the ability of the phosphorylated-Stat-3 SH2 domain-binding peptide, PY^{p}LKTK, to disrupt Stat-3 activity *in vitro*. The presence of PY^{p}LKTK, but not PYLKTK or PFLKTK, resulted in significant reduction in the levels of DNA-binding activities of Stat-3, and to a lesser extent of Stat-1, with no effect on that of Stat-5. Further analyses resulted in mapping the minimum active sequence to the tripeptide, XY^{p}L. The Stat-phosphopeptide was able to suppress transformation by the Src oncoprotein thereby proving the feasibility of use of such peptides in design of compounds that can target and neutralize aberrant Stat activity in tumors.

Shc-Ras-MAP kinase pathway

In addition to the activation of STATs, IL-3 as well as a number of other cytokines activate multiple signal transduction pathways, which include the Ras and PI3 kinase pathways. A brief description of these pathways is given below. Upon IL-3 stimulation, the adaptor molecule Shc is rapidly phosphorylated and associates with the phosphorylated β_c subunit of IL-3 (Sato *et al*., 1993; Pelicci *et al*., 1992; Blaikie *et al*., 1994). IL-3 stimulation also results in tyrosine phosphorylation of the inositol phosphatase (SHIP), which forms a complex with Shc, Grb2 and Sos, which are members of the Ras pathway (Lioubin *et al*., 1996). Upon recruitment, Shc binds to the phosphorylated Y577 of the β_c subunit and subsequently interacts with the adaptor protein GrB2, which in turn associates with mSos, which is the nucleotide exchange factor for Ras (Pratt *et al*., 1996; Rozakis-Adcock *et al*., 1993). This is followed by the activation of Ras and c-Raf (Pazdrak *et al*., 1995; Coffer *et al*., 1998a,b; Satoh *et al*., 1991). The activation of Ras and c-Raf results in downstream activation ERK1 and ERK2 that are members of the MAP kinase family (Pazdrak *et al*., 1995; Raines *et al*., 1992; Okuda *et al*., 1992; Welham *et al*., 1992; Coffer *et al*., 1998a,b). Activation of this cascade culminates in the increased expression of transcription factors c-*fos* and c-*jun* (Itoh *et al*., 1996; Satoh *et al*., 1991; Sato *et al*., 1993).

STAT proteins also serve as substrates for MAP kinases. The C-terminal domain of STATs that is required for transcriptional transactivation harbors a conserved serine residue. Phosphorylation of this serine residue in the TA domains of STAT-1, STAT-3, STAT-4 and STAT-5, by MAP kinases can enhance the transcriptional activity of STATs (Zhang *et al*., 1995; Wen *et al*., 1995; David *et al*., 1995a,b; Ihle, 1996).

In addition to activation of MAP kinase family members ERK1 and ERK2, IL-3 also activates another MAP kinase family member, p38 (Nagata *et al*., 1997a,b). Other members of the MAP kinase family that are activated by the IL-3/IL-5 and GM-CSF are the JNK/SAPK kinase proteins (de Groot *et al*., 1997; Terada *et al*., 1997; Nagata *et al*., 1997a,b; Foltz and Schrader, 1997; Liu *et al*., 1997). It has been proposed that SEK-1 may be involved in the activation of the JNK/SAPK pathway since IL-3 stimulation results in phosphorylation of SEK-1, at least in MC/9 cells (Foltz and Schrader, 1997). However, SEK-1 activation was not observed upon IL-3 stimulation of the myeloid cell line FD-CP2 (Nagata *et al*., 1997a,b). The precise mechanism of activation of these kinases is not entirely clear but there is evidence for the involvement of both the α and β_c chains of these receptors, specifically the importance of Y577 of the β_c chain and the kinase JAK2 (de Groot *et al*., 1997; Terada *et al*., 1997; Liu *et al*., 1997).

Recently, Lim and Cao (2001) reported that MEKK1 is involved in the regulation of Stat-3 activation by growth factors. They demonstrated that kinase-inactive MEKK1 inhibits Stat-3 phosphorylation on tyrosine and serine, and its transcriptional activity stimulated by epidermal growth factor and platelet-derived growth factor in different cell types. In contrast, active MEKK1 induced Stat-3 tyrosine and serine phosphorylation leading to a functionally active Stat-3 capable of binding DNA and enhancing transcription. Furthermore, they showed that Ser-727 is phosphorylated by MEKK1 *in vitro*, whereas Tyr-705 phosphorylation induced by MEKK1 involves Src and Janus kinases *in vivo*.

PI3K-PKB/AKT pathway

The IL-3/IL-5 and GM-CSF cytokines induce a rapid activation of the lipid kinase phosphatidyl inositol-3 kinase (PI3K) (Sato *et al*., 1993, 1994; Coffer *et al*., 1998a,b; Corey *et al*., 1993; Gold *et al*., 1994; al-Shami *et al*., 1997). Interestingly, the same region of the β_c chain that is responsible for the activation of the Ras pathway seems to be involved in signaling via the PI3K pathway (Sato *et al*., 1993). PI3K associates with the β_c chain and this interaction may be influenced by the adaptor protein p80. The p80 adaptor protein acts as a scaffold that interacts with the β_c chain and the p85 subunit of PI3 kinase in IL-3 stimulated cells (Rao and Mufson, 1995; Jucker and Feldman, 1995). This scaffold complex also contains members of the Src-kinase family, Yes and Lyn (Corey *et al*., 1993; Jucker and Feldman, 1995; Torigoe *et al*., 1992). Downstream proteins recruited by the PI3 kinase pathways upon IL-3 stimulation include the PKB/AKT protein. PI3K dependent activation of PKB/AKT in response to IL-3 stimulation has been observed in multiple cells lines and these studies suggest a role for this pathway in the transmission of cell survival signals in response to IL-3 (Coffer *et al*., 1998b; Tilton *et al*., 1997; Songyang *et al*., 1997; del Peso *et al*., 1997). Another downstream protein activated in response to IL-3 stimulation is p70S6 Kinase that also seems to mediate its effects via interaction with the β_c chain (Sato *et al*., 1993; Calvo *et al*., 1994). Another protein that feeds into the PI3K-PKB/AKT pathway is the Cbl protein, which also docks onto the adaptor protein Grb2 and Shc. Tyrosine phosphorylation of Cbl has been observed in IL-3 and GM-CSF stimulated cells which leads to activation of the PI3K pathway (Odai *et al*., 1995; Anderson *et al*., 1997; Chin *et al*., 1997; Sattler *et al*., 1996).

Anti-apoptotic pathways

One of the major phenotypic effects of IL-3 stimulation is its anti-apoptosis or cell survival function. All of the aforementioned pathways, the JAK/STAT pathway, the Shc-Ras-MAPK pathway and the PI3K-PKB/AKT pathways seem to play roles in this key function attributed to IL-3. Thus, overexpression of activated Ras (Ras-Val12) can abrogate the defects in IL-3 signaling and lead to cell survival emphasizing the importance of the Ras pathways in cell survival (Nicola *et al*., 1996; Terada *et al*., 1995). Also, apoptosis induced upon IL-3 withdrawal of 32Dcl3 and BaF3 cells can be overcome by overexpression of oncogenic c-Raf (Cleveland *et al*., 1994; Kinoshita *et al*., 1997). Similarly, expression of a dominant negative MAP kinase (MAPKK), which leads to loss of ERK activity, results in defects in IL-3 dependent growth and survival of BaF3 cells (Perkins *et al*., 1996).

The Bcl-2 gene family seems to mediate the cell survival or anti-apoptosis function of IL-3. In agreement with this, expression levels of Bcl-2 and Bcl-X_l are rapidly induced by IL-3, which seems to depend on Jak2 activation (Kinoshita *et al*., 1995a,b; Leverrier *et al*., 1997; Rinaudo *et al*., 1995; Sakai and Kraft, 1997). Also, overexpression of Bcl-2 or A1, a homologue of Bcl2, protects IL-3 dependent cells from apoptosis induced upon IL-3 withdrawal (Lin *et al*., 1996; Ito *et al*., 1997; Baffy *et al*., 1993). Several studies indicate that Ras-independent pathways also operate to bring about cell survival in response to IL-3. The PI3K-PKB/AKT pathway also plays a role in the anti-apoptosis function of IL-3. Overexpression of PKB/AKT, which leads to increased expression of c-*myc*, Bcl-2 and the Bcl-2 family member BAD, protects 32Dcl3 and BaF3 cells from IL-3 withdrawal mediated apoptosis whereas expression of non-functional PKB/AKT accelerates apoptosis due to IL-3 withdrawal (Songyang *et al*., 1997; del Peso *et al*., 1997; Ahmed *et al*., 1997; Datta *et al*., 1997).

Survivin, a member of the inhibitors-of-apoptosis gene family, is expressed in a cell-cycle-dependent manner in many common cancers but not in normal differentiated adult tissues. Carter *et al*. (2001) examined the survivin expression and regulation in acute myeloid leukemia (AML). Survivin was detected by Western blot analysis in all myeloid leukemia cell

lines and in 16 out of 18 primary AML samples tested. Interestingly, cytokine stimulation increased survivin expression in leukemic cell lines and in primary AML samples. Also, in cultured primary samples, single-cytokine stimulation substantially increased survivin expression in comparison with control cells, and the combination of G-CSF, GM-CSF, and SCF increased survivin levels even further. The authors also showed that the MEK inhibitor PD98059 down-regulated survivin expression in both resting and GM-CSF-stimulated OCI-AML3 cells, whereas the PI3K inhibitor LY294002 inhibited survivin expression only under conditions of GM-CSF stimulation. Thus, these results suggest that cytokines might exert their antiapoptotic and mitogenic effects, at least in part, by increasing survivin levels.

Negative regulation of JAK activity

Regulated JAK activation forms the fulcrum of the JAK-Src-STAT model. Therefore, cytokine receptor activation by JAKs triggers the downstream cascade of signaling events. Inappropriate activation of the receptors by JAKs can lead to a constitutive or de-regulated activation of downstream Src kinase that in turn could lead to de-regulated activation of STAT proteins and other transcription factors. In addition, the involvement of JAK kinases in multiple signal transduction cascades in response to a diverse group of cytokines and growth factors warrants a requirement of appropriate negative regulation of the JAK kinases. Such a negative regulatory mechanism may be necessary to preclude the possibility of constitutive activation of JAK kinases that may lead to an aberrant activation of downstream signals and inappropriate gene expression. Such a scenario is validated by observations that JAK kinase signaling has been implicated in disease states such as leukemia and other hematological malignancies (Leonard and O'Shea, 1998; Ward *et al*., 2000).

Involvement of proteasome-mediated degradation pathways

Proteasome mediated protein degradation pathways can modulate the activity of JAK kinases. This is consistent with the observation that degradation of STATs and cytokine/growth factor receptors is also seen in the regulation of signaling (Kim and Maniatis, 1996; Strous *et al*., 1996). Thus, it has been recently documented that proteasome inhibitors potentiate the IL-2 and IL-3 induced activation of the JAK kinase pathways (Callus and Mathey-Prevot, 1998; Yu and Burakoff, 1997). Yu and Burakoff (1997) showed that the proteasome inhibitor MG132 was able to stabilize the IL-2 induced tyrosine phosphorylation of Jak1 and Jak3. Also, treatment of IL-3 stimulated Ba/F3 with proteasome inhibitor, N-acetyl-L-leucinyl-L-leucinyl-norleucinal (LLnL), resulted in a stabilization of tyrosine phosphorylation of the IL-3 receptor-beta common chain, STAT-5, Shc, and mitogen activated protein kinases (MAPKs) (Callus and Mathey-Prevot, 1998). Furthermore, these authors showed that these stable phosphorylation events were the result of a prolonged activation of JAK1 and JAK2 kinases.

Negative regulation by Protein Tyrosine Phosphatases (PTPs)

Protein tyrosine phosphatases (PTPs) regulate the kinase activities of tyrosine kinases by de-phosphorylating tyrosine residues involved in catalytic function (Fischer *et al*., 1991; Pallen *et al*., 1992). Optimal activation of JAK kinases is positively regulated by phosphorylation of a critical tyrosine residue in the kinase-activating domain (Gauzzi *et al*., 1996; Feng *et al*., 1997; Zhou *et al*., 1997; Liu *et al*., 1997; Weiss and Schlessinger, 1998). Several studies have demonstrated the role of protein tyrosine phosphatases in the regulation of JAK signaling pathways (David *et al*., 1995a,b; Klingmuller *et al*., 1995; Yetter, 1995). PTPs such as SHP-1 have been shown to inhibit tyrosine phosphorylation of JAK kinases following their recruitment to receptor complexes that is facilitated by their binding to the receptors via their SH2 domains. These phosphatases have also been shown to bind JAK kinases directly and mediate their dephosphorylation (Weiss and Schlessinger, 1998; Haque *et al*., 1998; Migone *et al*., 1998; David *et al*., 1995a,b; Jiao *et al*., 1996). Regulation of JAK kinases by SH-PTP1 (also referred to as PTP1C, SHP, HCP, PTP1) has been demonstrated in signaling by interferons (David *et al*., 1995a,b). SH-PTP1 was also shown to be involved in inactivation of JAK2 and consequently abrogating the proliferative signals initiated by erythropoietin (Klingmuller *et al*., 1995). Similarly, PTPeC, has been shown to inhibit JAK kinase phosphorylation resulting in inhibition of differentiation and apoptosis in M1 cells stimulated by IL-6 and LIF (Tanuma *et al*., 2000). Bittorf *et al*. (1999) recently showed that SHP1 inhibited erythropoietin-induced erythroid differentiation and inhibition of apoptosis in an erythroleukemic cell line. This effect of SHP1 was a result of inhibition of both the JAK kinase and MAP kinase pathways. Also, You *et al*. (1999) recently documented an important role for SHP-2 in negative regulation of JAK kinases in interferon stimulated cells. Furthermore, Yin *et al*. (1997) performed a detailed molecular characterization of specific interactions between SHP-2 and JAK kinases. These authors demonstrated that SHP-2 is tyrosine phosphorylated by JAK1 and JAK2 but not JAK3, on Y304 and Y327 via direct association. The SHP-2 and JAK kinase association does not require the SH2 domain of SHP-2 or the kinase-like domain in JAKs but does require the N-terminal region of JAK proteins and regions encompassing amino acids residues 232 and 272 on the SHP-2 protein. Interestingly, SHP-2

phosphatase activity seems to be non-essential for JAK-SHP-2 interactions, as mutations that render SHP-2 phosphatase inactive do not abolish the interaction between SHP-2 and JAK kinases.

The transmembrane PTPase CD45 is highly expressed in all hematopoietic lineages at all stages of development and is a key regulator of antigen receptor signaling in T and B cells. Furthermore, Src-family kinases have been identified as primary molecular targets for CD45 and recently Irie-Sasaki *et al.* (2001) have shown that CD45 suppresses JAK (Janus kinase) kinases and negatively regulates cytokine receptor signaling. They demonstrated that targeted disruption of the cd45 gene leads to enhanced cytokine and interferon-receptor-mediated activation of JAKs and STAT proteins. *In vitro*, CD45 directly dephosphorylates and binds to JAKs and negatively regulates interleukin-3-mediated cellular proliferation and erythropoietin-dependent hematopoiesis. Myers *et al.* (2001) have shown that JAK2 and TYK2 are physiological substrates of PTP1B. Mouse embryo fibroblasts deficient in PTP1B displayed subtle changes in tyrosine phosphorylation, including hyperphosphorylation of JAK2 but not JAK1 suggesting that PTP1B may be an important physiological regulator of cytokine signaling.

The CIS/JAB/SOCS/SIS protein family modulates the activity of JAK signaling pathways

Recently, a new family of cytokine-inducible proteins has been characterized, that plays a critical role in negative regulation of cytokine signals processed by JAK kinases. These proteins, referred to as either CIS (cytokine-induced SH2 containing proteins) or SOCS (suppressors of cytokine signaling) or SIS (STAT-induced STAT inhibitor) or JAB (JAK-binding protein), possess SH2 domains that allow protein–protein interactions with members of the cytokine receptor family and other signaling components (Figure 2). For the sake of convenience, and taking into account the current confusing nomenclature, we will refer to these proteins as SOCS (suppressors of cytokine signaling) proteins. While a comprehensive analysis of this novel protein family is beyond the scope of this review, we present a brief narrative regarding the salient features of this important family and its plausible mode of action. For additional details readers are referred to several recent excellent reviews on the topic (Naka *et al.*, 1999; Yoshimura, 1998a,b; Nicholson and Hilton, 1998; Yasukawa *et al.*, 2000).

The SOCS proteins, which were cloned by several independent groups around the same time, are small proteins that possess SH2 domains and a conserved SOCS/CIS box (Naka *et al.*, 1997; Endo *et al.*, 1997; Starr *et al.*, 1997). The SOCS family, at the current time, is represented by at least eight members that play a critical role in the modulation of signals propagated by diverse cytokine receptors (Table 1; Naka *et al.*, 1999; Yoshimura, 1998a,b; Nicholson and Hilton, 1998; Yasukawa *et al.*, 2000). CIS1, the first identified member of the SOCS family, was cloned as an early response gene for IL-2, IL-3 and erythropoietin and was found to be associated with these receptors (Yoshimura *et al.*, 1995; Uchida *et al.*, 1997; Matsumoto *et al.*, 1997; Verdier *et al.*, 1998). Moreover, overexpression of CIS1 led to a suppression of signaling in response to IL-3 and Epo. SOCS1 (JAB or SSI1) was identified as a potent inhibitor of JAK kinases (Endo *et al.*, 1997). SOCS1 can interact with the JAK2 kinase domain and suppress IL-6 signal transduction pathways (Starr *et al.*, 1997). Recently, Sasaki *et al.* (2000) showed that SOCS3 suppresses erythropoietin mediated signaling pathways by binding to the Epo-receptor and JAK2. Deletion analysis experiments demonstrated that a cytoplasmic region of the Epo-R that contains the Y401 is responsible for SOCS3 binding and is required for optimal SOCS3 inhibitory activity. Furthermore, regions both N- and C-terminal to the SH2 domain of SOCS3 were necessary for binding to JAK2 and Epo-R.

Nicholson *et al.* (1999) also demonstrated that optimal activity of SOCS proteins and the consequential inhibition of cytokine activity requires both the SH2 and the N-terminal regions of the SOCS proteins. Deletion of the N-terminal 51–78 amino acid residues as well as mutations in the SH2 domains resulted in the prevention of inhibition of LIF signaling. Narazaki *et al.* (1998) mapped three distinct domains of the SOCS proteins that perform obligatory roles in inhibition of cytokine signaling. Mutation of the conserved C-terminal region of the SOCS proteins (SC-motif), the pre-SH2 and SH2 domains indicated that they were critical for the suppression of IL-6 signaling and collaborated in the mediation of inhibitory signals. Furthermore, they demonstrated that the pre-SH2 domain was crucial for SOCS functional activity; the SH2-domain was critical for interaction with JAK kinases and the SC-motif imparted stability to the SOCS proteins preventing them from proteasomal degradation.

Yasukawa *et al.* (1999) recently conducted further structure-function studies with a goal of understanding the mechanism of action of the SOCS proteins and found that at least one of the SOCS family members, SOCS1 (JAB/SSI1), inhibits JAK kinase activity by directly binding to the JAK kinase activation loop. SOCS1 specifically binds to the Y1007, that is part of the activation domain and is required for optimal JAK kinase activity. These authors also demonstrated that optimal JAK/SOCS binding required the SH2 domain of SOCS as well as the 12 amino acids region adjacent to the SH2 domain that house two residues (Ile68 and Leu75) that are conserved in SOCS proteins. High affinity binding and inhibition of the JAK2 kinase also requires an N-terminal 12 amino-acid region on SOCS1. Zhang *et al.* (1999) provided an alternate mode of action for the SOCS proteins that implicated the SOCS box domain in proteasomal degradation of target proteins. In this study, the authors showed that

Table 1 Tyrosine kinases, CIS/SOCS proteins and STATs activated by cytokine/growth factor signal transduction pathways

Cytokine	*JAK-Kinase*	*SOCS/CIS/JAB proteins*	*Non-JAK Kinase*	*STAT*
IL-2	Jak1, Jak2, Jak3	CIS1, CIS3, SOCS1	Fyn, Lck, Hck, Tec, Syk	Stat-5a, Stat-5b, Stat-3
IL-3	Jak2	CIS1, CIS2, CIS3, SOCS1	Lyn, Hck, Fyn	Stat-3, Stat-5a, Stat-5b
IL-4	Jak1, Jak3	CIS1, CIS2, CIS3	Lck, Tec	Stat-6
IL-5	Jak2	ND	Btk	Stat-1, Stat-3, Stat-5a, Stat-5b
IL-6	Jak1, Jak2, Tyk2	CIS1, CIS2, CIS3, SOCS1	Hck	Stat-1, Stat-3
IL-7	Jak1, Jak3	CIS1, CIS3	Lyn	Stat-5a, Stat-5b, Stat-3
IL-9	Jak3	ND	ND	Stat-5a, Stat-5b, Stat-3
IL-10	Jak1, Tyk2	CIS3	ND	Stat-1, Stat-3
IL-11	Jak1, Jak2, Tyk2	CIS3	Src, Yes	Stat-3
IL-12	Jak2, Tyk2	CIS1, CIS3	Lck	Stat-4
IL-13	Jak1, Jak2, Tyk2	CIS1, CIS3, SOCS1	Lsk	Stat-6
IL-15	Jak1, Jak3	ND	Lck	Stat-5a, Stat-5b, Stat-3
IFNα/β	Jak1, Tyk2	ND	Lck	Stat-1, Stat-2
IFNγ	Jak1, Jak2	CIS1, CIS2, CIS3, SOCS1	Hck, Lyn	Stat-1
Epo	Jak2	CIS1, CIS2, CIS3	Lyn	Stat-5a, Stat-5b
Tpo	Tyk2, Jak2	CIS1, CIS3	ND	Stat-5a, Stat-5b
G-CSF	Jak2, Jak3	CIS1, CIS2, CIS3	Lyn	Stat-3
GH	Jak2	CIS1, CIS2, CIS3, SOCS1	Src kinases	Stat-5a, Stat-5b, Stat-3
PRL	Jak2	CIS1, CIS2, CIS3, SOCS1	Src	Stat-5a, Stat-5b
Leptin	Jak2	CIS1, CIS3	ND	Stat-3, Stat-6, Stat-5a, Stat-5b
GM-CSF	Jak2	CIS1, CIS2, CIS3, SOCS1	Lyn, Hck	Stat-5a, Stat-5b
CNTF	Jak1, Jak2, Tyk2	ND	ND	Stat-3
CT-1	Jak1, Jak2, Tyk2	ND	ND	Stat-3
LIF	Jak1, Jak2, Tyk2	CIS1, CIS2, CIS3, SOCS1	Hck	Stat-3
OSM	Jak1, Jak2, Tyk2	ND	Src kinases	Stat-3
EGF	Jak1	ND	EGF-R, Src	Stat-1, Stat-3, Stat-5
PDGF	Jak1	ND	PDGF-R, Src	Stat-1, Stat-3, Stat-5
Insulin	Jak2	ND	IR, Src	Stat-1, Stat-5B

Note: ND = not determined

the SOCs box mediated interactions of SOCS family proteins with cytoskeletal proteins, elongin B and elongin C. The authors suggested that the SOCS/Elongin interaction might target the SOCS proteins and their associated substrates, which could be critical to propagate the cytokine responses, to proteasomal degradation pathways.

Thus, the CIS/JAB/SOCS/SIS family of proteins could regulate JAK signaling pathways by multiple mechanisms (Ward *et al*., 2000) (Figure 5). Some of these proteins such as JAB and CIS3 are able to bind to the kinase domain of JAK2 leading to the inhibition of its catalytic activity. As has been mentioned earlier, this interaction requires both the SH2 domain along with additional 12 N-terminal amino acid residues. This extended SH2 domain appears to bind the phospho-tyrosine residue Y1007 in the activation loop of JAK2, which is critical for the kinase activity of JAK2. Other members of this protein family seem to directly bind to receptors, where they may block the interaction of receptors with other signaling molecules. Alternatively, they might target the receptors and other interacting proteins for proteasomal degradation.

Iwamoto *et al*. (2000) investigated the effects of ectopic expression of JAB on v-Src-induced JAK-STAT activation. Forced expression of JAB in v-Src-transformed NIH3T3 cells neither suppressed phosphorylation of STAT-3 and JAK1/JAK2 nor blocked STAT-3-reporter gene activation. Also, colony forming assay showed that JAB did not suppress v-Src-induced transformation of NIH3T3 cells, whereas dominant negative STAT-3 suppressed it. However, JAB could downregulate phosphorylation of STAT-1 and STAT-3 induced by interferon gamma (IFNgamma) and interleukin-6 (IL-6) plus the soluble IL-6 receptor (sIL-6R), respectively. In addition, *in vitro* kinase assay indicated that JAB suppressed hyperactivation of JAK1/JAK2 and JAK1 induced by IFNgamma and IL-6 plus sIL-6R respectively, but not v-Src-induced basal JAK1/JAK2 activity. Interestingly, JAK1 and JAK2 activated by either v-Src or IL-6 could bind JAB, but JAB could only inhibit IL-6 mediated STAT activation, suggesting that JAB distinguishes cytokine-induced JAK-STAT signaling from v-Src-induced signals as it cannot suppress the transformation process mediated by v-Src.

JAKs as mediators of apoptosis signals

Observations that JAK kinases mediate proliferative responses from a variety of cytokines/growth factors is indicative of their indispensability to the overall growth stimulus provided by the cytokine/growth factor signal transduction pathways. This observation also suggests that JAK kinase activity may play an important role in the prevention of cell death or apoptosis. JAK kinase activity, in conjunction with other pathways that dictate proliferation such as the Ras, PI3K, MAPK pathways, may contribute to the overall proliferative stimulus. On the other hand, JAK kinase activity may also have an underlying influence on the levels of cell

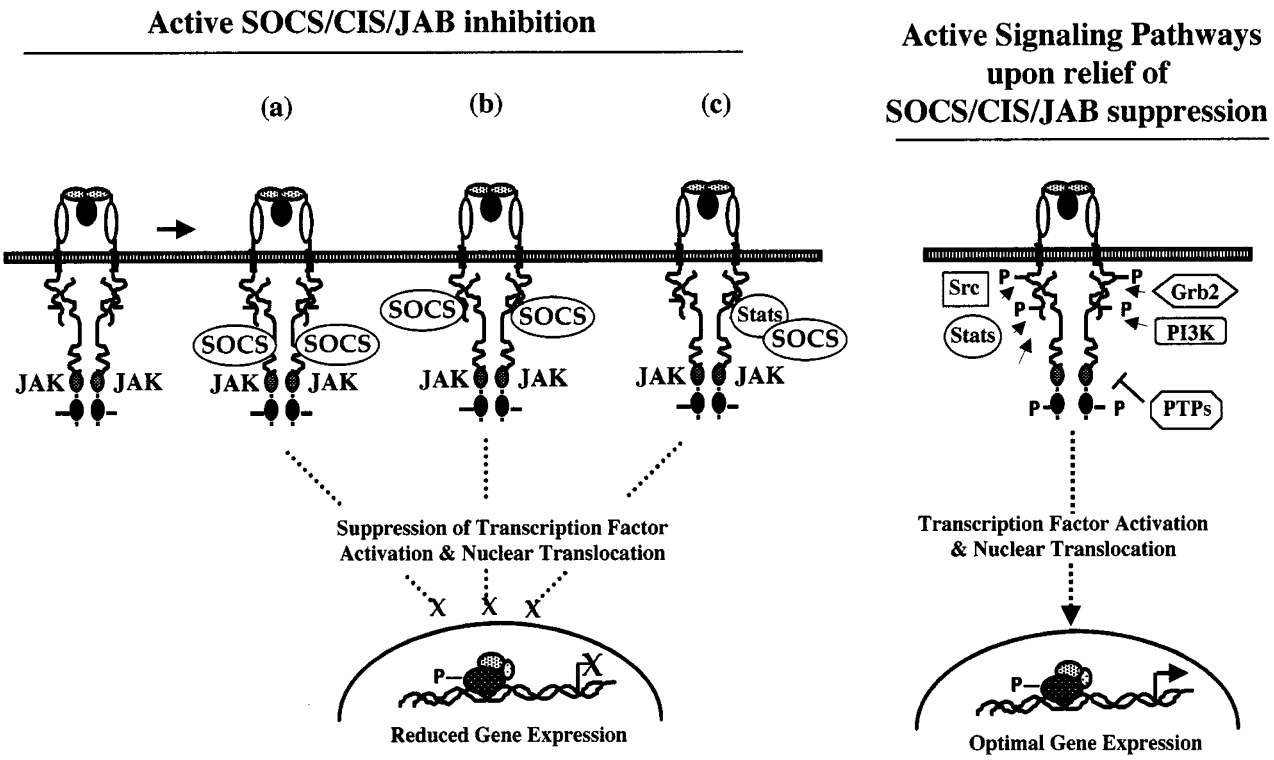

Figure 5 Pathways of signal suppression by SOCS/CIS/JAB proteins. Active SOCS/CIS/JAB suppression of signal transduction can be achieved by (**a**) SOCS proteins binding to activation loops in JAK kinases that prevents the access of substrates and/or ATP to the JAK catalytic pocket; or (**b**) SOCS proteins can bind to JAK-proximal sites on cytokine receptors and suppress JAK catalytic activity; or (**c**) SOCS proteins can either bind to STATs or prevent STAT binding to receptors. Either of these mechanisms of kinase inhibition can result in signal suppression. Relief of the SOCS/CIS/JAB protein suppression by inactivation or degradation of SOCS/CIS/JAB proteins allows signal transduction

survival and apoptosis. Several recent studies that implicate JAK kinase activity in the modulation of rates of cell survival and apoptosis have corroborated this hypothesis.

Proteins represented by the Bcl-2 family genes modulate the levels of apoptosis and cell survival by either positively or negatively influencing the cell death and cell survival machinery (Antonsson and Martinou, 2000; Pellegrini and Strasser, 1999; Reed, 1997; Adams and Cory, 1998). Thus, the family members Bcl-2, Bcl-XL and Mcl-1 inhibit apoptosis whereas Bax, Bad and Bak proteins accelerate apoptosis. Signaling cascades emanating from diverse pathways regulate the level of expression of Bcl-2 family members. Packham *et al.* (1998) recently implicated the JAK signaling pathway in the modulation of cell survival and apoptosis by regulating members of the Bcl-2 family. This study demonstrated that, regulation of the cell death effector Bcl-XL is mediated by the JAK kinase pathway. Moreover, the authors portray the specificity of the role of JAK kinases by providing evidence that regulation of Bcl-XL protein levels was independent of the status of other signaling components such as STAT proteins, PI-3 kinases and Ras. The same group also highlighted the role of JAK kinase signaling in mediating the rescue of p53-dependent cell cycle arrest and apoptosis in cytokine treated cells (Quelle *et al.*, 1998). This study evaluated the role of signal transduction pathways in gamma-radiation (IR) induced p53 dependent apoptosis and p53-independent cell cycle arrest. The authors showed that the IR-induced cell cycle arrest and apoptosis was inhibited by cytokines such as erythropoietin and that these effects were dependent on the activation of JAK kinases. Using mutants of the erythropoietin receptors (Epo-R) this study delineated the functional domains on the Epo-R that were responsible for mediating the cell cycle arrest and apoptosis signals. While the membrane proximal domain of the Epo-R was sufficient to prevent IR-induced cell death, the membrane distal domain was required for subverting the growth arrest associated with IR-induced DNA damage. Furthermore, that the activation of JAK kinase signaling was necessary and sufficient for these signals was underscored by the observation that cell survival by Epo was not dependent on the activation of other signaling pathways such as the PI3-Kinase, PLC-gamma, Ras or STAT pathways. JAK kinase mediated cell survival, however, required the participation of Bcl-2 family members such as Bcl-XL. Sakai and Kraft (1997) also illustrated the role of the JAK2 kinase domain in induction of Bcl-2 protein that mediated cell survival and delay of hematopoietic cell death.

Implication of aberrant JAK activity in disease states

Mutations in JAK3 predispose to certain forms of severe combined immunodeficiency syndrome (SCID)

Mutations in the γ_c chain of the IL-2 receptor have been known to be responsible for the X-linked severe combined immunodeficiency syndrome, X-SCID (O'Shea *et al*., 1997). X-SCID is an inherited disorder that is typified by a rampant defect in the body's immune system. γ_c chain specifically associates with JAK3 kinase and therefore it was postulated that mutations in JAK3 might predispose to some form of SCID (Russell *et al*., 1994; Noguchi *et al*., 1993; Leonard, 1996; O'Shea *et al*., 1997). In confirmation of this hypothesis, several patients with autosomal recessive SCID have been identified, that harbor mutations in their JAK3 locus (Candotti *et al*., 1997; Russell *et al*., 1995; Macchi *et al*., 1995; Cacalano *et al*., 1999). Furthermore, to elucidate the role of JAK3 in development several groups engineered JAK3 deficient mice (Thomis *et al*., 1995; Nosaka *et al*., 1995; Park *et al*., 1995). JAK3 nullizygous mice are immunodeficient, similar to γ_c-deficient mice (Cao *et al*., 1995; DiSanto *et al*., 1995). These mice have severely depleted B-cell repertoire and a slight increase in peripheral T-cells. This observation is in contrast to that observed in human SCID patients with mutations in JAK3 and the γ_c, who exhibit severely depleted T-cells with normal or slightly increased numbers of B cells. The observed defect in lymphoid development is, at least in part, due to lack of IL-7 signal transduction. Cacalano *et al*. (1999) reported a single Y100C amino acid substitution in the N-terminus JH7 domain of JAK3 that was identified in a patient with autosomal severe combined immunodeficiency (SCID). IL-2 responsive signaling was compromised in B-cell lines derived from patient cells. Furthermore, the authors demonstrated that a region encompassing the JH6 and JH7 domains of JAK3 was sufficient for interaction of the kinase with the proline-rich Box 1 region of the IL-2 receptor and was sufficient in reconstituting the IL-2 dependent response.

Aberrant activation of JAK kinases in leukemia

Aberrant activation of JAK kinase activity has been implicated in several hematological malignancies (Ward *et al*., 2000). Thus, a t(9;12)(p24;p13) chromosomal translocation has been detected in a patient with T cell childhood acute lymphoblastic leukemia (Lacronique *et al*., 1997, 2000). This translocation results in the fusion of the C-terminal kinase catalytic region of JAK2 to the N-terminal region of the ETS-transcription family member, TEL, to generate an overactive TEL-JAK2 tyrosine kinase that could impart cytokine independence to an IL-3 dependent cell line, Ba/F3. Peeters *et al*. (1997) detected similar translocations involving the JAK2 kinase at 9p24 and TEL (the ETV6 gene at 12p13) in both myeloid and lymphoid leukemias. A t(9;12)(p24;p13) translocation was detected in a case of early pre-B acute lymphoid leukemia and a t(9;15;12)(p24;q15;p13) translocation was detected in atypical chronic myelogenous leukemia. In either case the authors detected different fusion mRNA transcripts and one fusion protein product that consisted of the helix–loop–helix (HLH) domain of the TEL (ETV6) gene and the tyrosine kinase domain of JAK2. A Glycine 341 to Glutamic acid amino acid substitution in the *Drosophila hopscotch* gene was shown to cause leukemia-like hematopoietic defect (Luo *et al*., 1995; Harrison *et al*., 1995). The only known *Drosophila* member of the JAK kinase family was identified during the cloning of *hopscotch* (Binari and Perrimon, 1994). *Hopscotch* is required maternally for the establishment of the normal array of embryonic segments in *Drosophila*. It was observed that the *hopscotch* gene was involved in the control of pair-rule gene transcription in a stripe-specific manner thereby providing the first evidence for stripe-specific regulation of pair-rule genes by a tyrosine kinase. Furthermore, a Glycine 341 to Glutamic acid amino acid substitution in the *hopscotch* gene was shown to cause leukemia-like hematopoietic defects (Luo *et al*., 1995; Harrison *et al*., 1995). This study was the first study, which indicated that a mutant JAK kinase could cause leukemia-like abnormalities. These studies, taken together, indicate that aberrant activation of JAK kinases can result in hematological abnormalities. The role of JAK kinases in development and disease have been examined using gene targeting approaches in mice (Rodig *et al*., 1998; Neubauer *et al*., 1998; Parganas *et al*., 1998; Park *et al*., 1995; Thomis *et al*., 1995; Nosaka *et al*., 1995). Mice carrying a disruption of the JAK1 locus are susceptible to perinatal lethality primarily due to defective suckling arising from neurological deficits (Rodig *et al*., 1998). Furthermore, JAK1 deficient cells derived from these mice are defective in signaling via multiple cytokine receptors families such as the gp130 family, the interferon receptor family and gamma chain containing receptors. Mice deficient in JAK2 succumb to embryonic lethality and die at embryonic day E12.5 (Neubauer *et al*., 1998; Parganas *et al*., 1998). The embryonic lethality is mostly due to defects in erythropoiesis and cells from JAK2 nullizygous mice have defective signaling response to cytokines from the IL-3, interferon, and single chain (excluding G-CSFR) receptor families. JAK3 deficient mice exhibit severe defects in lymphoid development and show aberrations in B-cell maturation and T-lymphocyte activation (Park *et al*., 1995; Thomis *et al*., 1995; Nosaka *et al*., 1995). Apart from the defects in B cell and T-cell development, JAK3 deficient mice also show defects in the development of natural killer (NK) cells. Moreover, Grossman *et al*. (1999) recently demonstrated that JAK3 nullizygous mice display dysregulated myelopoiesis. Their results demonstrate that JAK3 deficient mice show evidence of increased immature neutrophil and monocyte counts in peripheral blood smears along with splenomegaly. Specific cell-surface marker analysis also indicated an expansion of cells of the myeloid

lineage. Taken together, these observations indicate that JAK3 has an important role in the development of cells destined for both the myeloid and lymphoid lineages. Further detailed analysis of the role of JAK3 in cells of lymphoid and myeloid origin may yield important clues regarding the role of JAK3 in the development of these cell lineages.

Perspective and future directions

It is becoming increasingly clear that the interaction of cytokines with their receptors leads to the activation of multiple signal transduction pathways, which are very similar for several cytokines. However, each cytokine appears to elicit different biochemical and biological responses from a given cell. These responses appear to be different for different cytokines, even when they share the same signal transducing receptor unit. This becomes very apparent, when one examines the nature of biological response elicited by IL-3 and GMCSF, both of which share the same β-chain. It is therefore reasonable to assume that different cytokines elicit a different combination of responses and these responses are dependent on the nature of their receptor components (such as the alpha chains) as well as the nature of cellular factors that are present in a given cell type. The nature of the downstream signaling proteins recruited by these receptors account for the functional pleiotropy as well as redundancy of cytokine stimulus. A complete understanding of the subtle differences that exist between different cytokine responses and the cross talk between different members of these signal transduction pathways will provide a better understanding of the mechanisms that are involved in the regulation of cell growth, differentiation and survival.

A reasonable degree of clinical success has been obtained using recombinant hematopoietic growth factors produced in mammalian cell lines and in microbial fermentation processes. However, these proteins are expensive to produce, require parenteral administration, and in some cases have provoked detrimental immune responses. Several investigators have utilized the high throughput biological function and receptor binding assays to screen randomly produced organic compounds and short peptides to identify small molecular mimetics and antagonists of hematopoietic growth factors such as erythropoietin, granulocyte colony-stimulating factor, and thrombopoietin. The structural study of mimetic-receptor complexes has allowed analysis of molecular details of growth factor-induced receptor activation may yield new insights into the molecular basis of hematopoietic signal transduction.

While we have learnt a great deal about the role of JAK kinases in signaling, a number of questions about these kinases remain unanswered. For example, the role of the unique structural domains (JH3–JH7) present in these kinases is yet to be deciphered. Clearly, these domains play a critical role in protein–protein interactions and understanding the nature of proteins that interact with these domains is likely to further our understanding of the role played by these kinases in cell signaling. Like other growth factor/receptor interactions, cytokine/receptor interactions lead to the formation of multi-protein complexes which includes JAKs, Src family kinases, STATs, Ras, MAP and PI3Kinase family of proteins, protein phosphatases as well as CIS/JAB/SOCS family of proteins. It is intriguing to note that different cytokine and interferon receptors interact with very similar sets of signaling molecules such as JAKs, STATs, serine/threonine kinases, dual kinases, phosphatases and their negative regulators and yet each cytokine elicits very distinctive biological and biochemical responses from a given cell. It is clear that the exact stoichiometry of the complexes that are formed following cytokine/receptor interactions plays a critical role in eliciting these distinctive responses.

The observation that different cytokines and interferons activate similar STAT complexes that bind to very related sequences and yet elicit very different biological responses raises the question as to how specific gene expression is achieved. It is possible that STATs themselves do not dictate the phenotype that results from cytokine/interferon stimulation. It is likely, that it is the combination of different pathways that lead to the activation of different transcription factors which act in concert with STATs that dictate the phenotype produced by a given cytokine/receptor interaction. It is clear that JAK and Src kinases play a critical role in the activation of these multiple pathways and a detailed understanding of the mode of action of JAK kinases is likely to lead to a better understanding of the complex biological processes regulated by interleukins, interferons and other growth hormones.

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References

Adams JM and Cory S. (1998). *Science,* **281,** 1322–1326.
Ahmed NN, Grimes HL, Bellacosa A, Chan TO and Tsichlis PN. (1997). *Proc. Natl. Acad. Sci. USA,* **94,** 3627–3632.
Akira S, Taga T and Kishimoto T. (1993). *Adv. Immunol.,* **54,** 1–78.
al-Shami A, Bourgoin SG and Naccache PH. (1997). *Blood,* **89,** 1035–1044.
Anderson SM, Burton EA and Koch BL. (1997). *J. Biol. Chem.,* **272,** 739–745.
Antonsson B and Martinou JC. (2000). *Exp. Cell Res.,* **256,** 50–57.
Azam M, Erdjument-Bromage H, Kreider BL, Xia M, Quelle F, Basu R, Saris C, Tempst P, Ihle JN and Schindler C. (1995). *EMBO J.,* **14,** 1402–1411.

Baffy G, Miyashita T, Williamson JR and Reed JC. (1993). *J. Biol. Chem.,* **268,** 6511–6519.
Bagley CJ, Woodcock JM, Stomski FC and Lopez AF. (1997). *Blood,* **89,** 1471–1482.
Barber DL, Beattie BK, Mason JM, Nguyen MH, Yoakim M, Neel BG, D'Andrea AD and Frank DA. (2001). *Blood,* **97,** 2230–2237.
Barry SC, Korpelainen E, Sun Q, Stomski FC, Moretti PA, Wakao H, D'Andrea RJ, Vadas MA, Lopez AF and Goodall GJ. (1997). *Blood,* **89,** 842–852.
Binari R and Perrimon N. (1994). *Genes Dev.,* **8,** 300–312.
Bittorf T, Seiler J, Zhang Z, Jaster R and Brock J. (1999). *Biol. Chem.,* **380,** 1201–1209.
Blaikie P, Immanuel D, Wu J, Li N, Yajnik V and Margolis B. (1994). *J. Biol. Chem.,* **269,** 32031–32034.
Blalock WL, Weinstein-Oppenheimer C, Chang F, Hoyle PE, Wang XY, Algate PA, Franklin RA, Oberhaus SM, Steelman LS and McCubrey JA. (1999). *Leukemia,* **13,** 1109–1166.
Bowman T, Broome MA, Sinibaldi D, Wharton W, Pledger WJ, Sedivy JM, Irby R, Yeatman T, Courtneidge SA and Jove R. (2001). *Proc. Natl. Acad. Sci. USA,* **98,** 7319–7324.
Bromberg J and Chen X. (2001). 'STAT proteins: signal tranducers and activators of transcription.' *Meth. Enzymol.,* **333,** 138–151.
Cacalano NA, Migone TS, Bazan F, Hanson EP, Chen M, Candotti F, O'Shea JJ and Johnston JA. (1999). *EMBO J.,* **18,** 1549–1558.
Caldenhoven E, van Dijk T, Raaijmakers JA, Lammers JW, Koenderman L and De Groot RP. (1995). *J. Biol. Chem.,* **270,** 25778–25784.
Callus BA and Mathey-Prevot B. (1998). *Blood,* **91,** 3182–3192.
Calvo V, Wood M, Gjertson C, Vik T and Bierer BE. (1994). *Eur. J. Immunol.,* **24,** 2664–2671.
Candotti F, Oakes SA, Johnston JA, Giliani S, Schumacher RF, Mella P, Fiorini M, Ugazio AG, Badolato R, Notarangelo LD, Bozzi F, Macchi P, Strina D, Vezzoni P, Blaese RM, O'Shea JJ and Villa A. (1997). *Blood,* **90,** 3996–4003.
Cantor AB and Orkin SH. (2001). *Curr. Opin. Genet. Dev.,* **11,** 513–519.
Cao X, Shores EW, Hu-Li J, Anver MR, Kelsall BL, Russell SM, Drago J, Noguchi M, Grinberg A and Bloom ET. (1995). *Immunity,* **2,** 223–238.
Cao X, Tay A, Guy GR and Tan YH. (1996). *Mol. Cell. Biol.,* **16,** 1595–1603.
Carlesso N, Frank DA and Griffin JD. (1996). *J. Exp. Med.,* **183,** 811–820.
Carter BZ, Milella M, Altieri DC and Andreeff M. (2001). *Blood,* **97,** 2784–2790.
Chaturvedi P, Reddy MV and Reddy EP. (1998). *Oncogene,* **16,** 1749–1758.
Chaturvedi P, Sharma S and Reddy EP. (1997). *Mol. Cell. Biol.,* **17,** 3295–3304.
Chen X, Vinkemeier U, Zhao Y, Jeruzalmi D, Darnell Jr JE, Kuriyan J. (1998). *Cell,* **93,** 827–839.
Chen M, Cheng A, Chen YQ, Hymel A, Hanson EP, Kimmel L, Minami Y, Taniguchi T, Changelian PS and O'Shea JJ. (1997). *Proc. Natl. Acad. Sci. USA,* **94,** 6910–6915.
Chin H, Saito T, Arai A, Yamamoto K, Kamiyama R, Miyasaka N and Miura O. (1997). *Biochem. Biophys. Res. Commun,* **239,** 412–417.
Chin YE, Kitagawa M, Su WC, You ZH, Iwamoto Y and Fu XY. (1996). *Science,* **272,** 719–722.
Cleveland JL, Troppmair J, Packham G, Askew DS, Lloyd P, Gonzalez-Garcia M, Nunez G, Ihle JN and Rapp UR. (1994). *Oncogene,* **9,** 2217–2226.
Coffer PJ, Geijsen N, M'Rabet L, Schweizer RC, Maikoe T, Raaijmakers JA, Lammers JW and Koenderman L. (1998a). *Biochem. J.,* **329,** 121–130.
Coffer PJ, Schweizer RC, Dubois GR, Maikoe T, Lammers JW and Koenderman L. (1998b). *Blood,* **91,** 2547–2557.
Colamonici O, Yan H, Domanski P, Handa R, Smalley D, Mullersman J, Witte M, Krishnan K and Krolewski J. (1994a). *Mol. Cell. Biol.,* **14,** 8133–8142.
Colamonici OR, Uyttendaele H, Domanski P, Yan H and Krolewski JJ. (1994b). *J. Biol. Chem.,* **269,** 3518–3522.
Cook WD, Matcalf D, Nicola NA, Burgess AW and Walker F. (1985). *Cell,* **41,** 677–683.
Cooper JA and Howell B. (1993). *Cell,* **73,** 1051–1054.
Copeland NG, Gilbert DJ, Schindler C, Zhong Z, Wen Z, Darnell Jr JE, Mui AL, Miyajima A, Quelle FW, Ihle JN and Jenkins NA. (1995). *Genomics,* **29,** 225–228.
Corey S, Eguinoa A, Puyana-Theall K, Bolen JB, Cantley L, Mollinedo F, Jackson TR, Hawkins PT and Stephens LR. (1993). *EMBO J.,* **12,** 2681–2690.
Cornelis S, Fache I, Van der Heyden J, Guisez Y, Tavernier J, Devos R, Fiers W and Plaetinck G. (1995). *Eur. J. Immunol.,* **25,** 1857–1864.
Danial NN, Losman JA, Lu T, Yip N, Krishnan K, Krolewski J, Goff SP, Wang JY and Rothman PB. (1998). *Mol. Cell. Biol.,* **18,** 6795–6804.
Danial NN, Pernis A and Rothman PB. (1995). *Science,* **269,** 1875–1877.
Darnell Jr JE. (1997). *Science,* **277,** 1630–1635.
Darnell Jr JE. (1998). *J. Interferon Cytokine Res.,* **18,** 549–554.
Darnell Jr JE, Kerr IM and Stark GR. (1994). *Science,* **264,** 1415–1421.
DaSilva L, Howard OM, Rui H, Kirken RA and Farrar WL. (1994). *J. Biol. Chem.,* **269,** 18267–18270.
Datta SR, Dudek H, Tao X, Masters S, Fu H, Gotoh Y and Greenberg ME. (1997). *Cell,* **91,** 231–241.
David M, Chen HE, Goelz S, Larner AC and Neel BG. (1995a). *Mol. Cell. Biol.,* **15,** 7050–7058.
David M, Petricoin III E, Benjamin C, Pine R, Weber MJ and Larner AC. (1995b). *Science,* **269,** 1721–1723.
de Groot RP, Coffer PJ and Koenderman L. (1998). *Cell Signal,* **10,** 619–628.
de Groot RP, van Dijk TB, Caldenhoven E, Coffer PJ, Raaijmakers JA, Lammers JWJ and Koenderman L. (1997). *J. Biol. Chem.,* **272,** 2319–2325.
del Peso L, Gonzalez-Garcia M, Page C, Herrera R and Nunez G. (1997). *Science,* **278,** 687–689.
DiSanto JP, Muller W, Guy-Grand D, Fischer A and Rajewsky K. (1995). *Proc. Natl. Acad. Sci. USA,* **92,** 377–381.
Duhe RJ and Farrar WL. (1995). *J. Biol. Chem.,* **270,** 23084–23089.
Durstin M, Inhorn RC and Griffin JD. (1996). *J. Immunol.,* **157,** 534–540.
Eder M, Ernst TJ, Ganser A, Jubinsky PT, Inhorn R, Hoelzer D and Griffin JD. (1994). *J. Biol. Chem.,* **269,** 30173–30180.
Eilers A, Kanda K, Klose B, Krolewski J and Decker T. (1996). *Cell Growth Differ.,* **7,** 833–840.

Endo TA, Masuhara M, Yokouchi M, Suzuki R, Sakamoto H, Mitsui K, Matsumoto A, Tanimura S, Ohtsubo M, Misawa H, Miyazaki T, Leonor N, Taniguchi T, Fujita T, Kanakura Y, Komiya S and Yoshimura A. (1997). *Nature,* **387,** 921–924.

Feng J, Witthuhn BA, Matsuda T, Kohlhuber F, Kerr IM and Ihle JN. (1997). *Mol. Cell. Biol.,* **17,** 2497–2501.

Fischer EH, Charbonneau H and Tonks NK. (1991). *Science,* **253,** 401–406.

Flores-Morales A, Pircher TJ, Silvennoinen O, Gustafsson JA, Sanchez-Gomez M, Norstedt G, Haldosen LA and Wood TJ. (1998). *Mol. Cell. Endocrinol.,* **138,** 1–10.

Foltz IN and Schrader JW. (1997). *Blood,* **89,** 3092–3096.

Frank DA and Varticovski L. (1996). *Leukemia,* **10,** 1724–1730.

Frank SJ, Yi W, Zhao Y, Goldsmith JF, Gilliland G, Jiang J, Sakai I and Kraft AS. (1995). *J. Biol. Chem.,* **270,** 14776–14785.

Garcia R, Bowman TL, Niu G, Yu H, Minton S, Muro-Cacho CA, Cox CE, Falcone R, Fairclough R, Parsons S, Laudano A, Gazit A, Levitzki A, Kraker A and Jove R. (2001). *Oncogene,* **20,** 2499–2513.

Gauzzi MC, Barbieri G, Richter MF, Uze G, Ling L, Fellous M and Pellegrini S. (1997). *Proc. Natl. Acad. Sci. USA,* **94,** 11839–11844.

Gauzzi MC, Velazquez L, McKendry R, Mogensen KE, Fellous M and Pellegrini S. (1996). *J. Biol. Chem.,* **271,** 20494–20500.

Gearing DP, King JA, Gough NM and Nicola NA. (1989). *EMBO J.,* **8,** 3667–3676.

Gold MR, Duronio V, Saxena SP, Schrader JW and Aebersold R. (1994). *J. Biol. Chem.,* **269,** 5403–5412.

Goujon L, Allevato G, Simonin G, Paquereau L, Le Cam A, Clark J, Nielsen JH, Djiane J, Postel-Vinay MC, Edery M and Kelly PA. (1994). *Proc. Natl. Acad. Sci. USA,* **91,** 957–961.

Grossman WJ, Verbsky JW, Yang L, Berg LJ, Fields LE, Chaplin DD and Ratner L. (1999). *Blood,* **94,** 932–939.

Hackett RH, Wang YD and Larner AC. (1995). *J. Biol. Chem.,* **270,** 21326–21330.

Hanks SK, Quinn AM and Hunter T. (1988). *Science,* **241,** 42–52.

Haque SJ, Harbor P, Tabrizi M, Yi T and Williams BR. (1998). *J. Biol. Chem.,* **273,** 33893–33896.

Harrison DA, Binari R, Nahreini TS, Gilman M and Perrimon N. (1995). *EMBO J.,* **14,** 2857–2865.

Hayashida K, Kitamura T, Gorman DM, Arai K, Yokota T and Miyajima A. (1990). *Proc. Natl. Acad. Sci. USA,* **87,** 9655–9659.

Heim MH. (1999). *J. Recept. Signal Transduct. Res.,* **19,** 75–120.

Horvath CM, Wen Z and Darnell Jr JE. (1995). *Genes Dev.,* **9,** 984–994.

Hunter T. (1993). *Nature,* **366,** 114–116.

Ihle JN. (1996). *BioEssays,* **18,** 95–98.

Ihle JN, Nosaka T, Thierfelder W, Quelle FW and Shimoda K. (1997). *Stem Cells,* **15,** 105–111; discussion 112.

Ihle JN, Witthuhn BA, Quelle FW, Yamamoto K and Silvennoinen O. (1995). *Annu. Rev. Immunol.,* **13,** 369–398.

Ilaria Jr RL and Van Etten RA. (1996). *J. Biol. Chem.,* **271,** 31704–31710.

Inhorn RC, Carlesso N, Durstin M, Frank DA and Griffin JD. (1995). *Proc. Natl. Acad. Sci. USA,* **92,** 8665–8669.

Irie-Sasaki J, Sasaki T, Matsumoto W, Opavsky A, Cheng M, Welstead G, Griffiths E, Krawczyk C, Richardson CD, Aitken K, Iscove N, Koretzky G, Johnson P, Liu P, Rothstein DM and Penninger JM. (2001). *Nature,* **409,** 349–354.

Ito T, Deng X, Carr B and May WS. (1997). *J. Biol. Chem.,* **272,** 11671–11673.

Itoh N, Yonehara S, Schreurs J, Gorman DM, Maruyama K, Ishii A, Yahara I, Arai K and Miyajima A. (1990). *Science,* **247,** 324–327.

Itoh T, Muto A, Watanabe S, Miyajima A, Yokota T and Arai K. (1996). *J. Biol. Chem.,* **271,** 7587–7592.

Iwamoto T, Senga T, Naito Y, Matsuda S, Miyake Y, Yoshimura A and Hamaguchi M. (2000). *Oncogene,* **19,** 4795–4801.

Jaster R, Zhu Y, Pless M, Bhattacharya S, Mathey-Prevot B and D'Andrea AD. (1997). *Mol. Cell. Biol.,* **17,** 3364–3372.

Jiang N, He TC, Miyajima A and Wojchowski DM. (1996). *J. Biol. Chem.,* **271,** 16472–16476.

Jiao H, Berrada K, Yang W, Tabrizi M, Platanias LC and Yi T. (1996). *Mol. Cell. Biol.,* **16,** 6985–6992.

Jubinsky PT, Nathan DG, Wilson DJ and Sieff CA. (1993). *Blood,* **81,** 587–591.

Jucker M and Feldman RA. (1995). *J. Biol. Chem.,* **270,** 27817–27822.

Kazansky AV and Rosen JM. (2001). *Cell Growth Differ.,* **12:** 1–7.

Kazansky AV, Kabotyanski EB, Wyszomierski SL, Mancini MA and Rosen JM. (1999). *J. Biol. Chem.,* **274,** 22484–22492.

Kim TK and Maniatis T. (1996). *Science,* **273,** 1717–1719.

Kinoshita T, Shirouzu M, Kamiya A, Hashimoto K, Yokoyama S and Miyajima A. (1997). *Oncogene,* **15,** 619–627.

Kinoshita T, Yokota T, Arai K and Miyajima A. (1995a). *Oncogene,* **10,** 2207–2212.

Kinoshita T, Yokota T, Arai K and Miyajima A. (1995b). *EMBO J.,* **14,** 266–275.

Kishimoto T. (1989). *Blood,* **74,** 1–10.

Kishimoto T, Akira S and Taga T. (1992). *Science,* **258,** 593–597.

Kishimoto T, Taga T and Akira S. (1994). *Cell,* **76,** 253–262.

Klingmuller U, Lorenz U, Cantley LC, Neel BG and Lodish HF. (1995). *Cell,* **80,** 729–738.

Kohlhuber F, Rogers NC, Watling D, Feng J, Guschin D, Briscoe J, Witthuhn BA, Kotenko SV, Pestka S, Stark GR, IhleJN and Kerr IM. (1997). *Mol. Cell. Biol.,* **17,** 695–706.

Kotenko SV, Izotova LS, Pollack BP, Muthukumaran G, Paukku K, Silvennoinen O, Ihle JN and Pestka S. (1996). *J. Biol. Chem.,* **271,** 17174–17182.

Kouro T, Kikuchi Y, Kanazawa H, Hirokawa K, Harada N, Shiiba M, Wakao H, Takaki S and Takatsu K. (1996). *Int. Immunol.,* **8,** 237–245.

Kovarik P, Mangold M, Ramsauer K, Heidari H, Steinborn R, Zotter A, Levy DE, Muller M and Decker T. (2001) *EMBO J.,* **20,** 91–100.

Kreider BL, Phillips PD, Prystowsky MB, Shirsat N, Pierce JH, Tushinski R and Rovera G. (1990). *Mol. Cell. Biol.,* **10,** 4846–4853.

Krishnan K, Pine R and Krolewski JJ. (1997). *Eur. J. Biochem.,* **247,** 298–305.

Kruger A and Anderson SM. (1991). *Oncogene,* **6,** 245–256.

Lacronique V, Boureux A, Monni R, Dumon S, Mauchauffe M, Mayeux P, Gouilleux F, Berger R, Gisselbrecht S, Ghysdael J and Bernard OA. (2000). *Blood,* **95,** 2076–2083.

Lacronique V, Boureux A, Valle VD, Poirel H, Quang CT, Mauchauffe M, Berthou C, Lessard M, Berger R, Ghysdael J and Bernard OA. (1997). *Science,* **278,** 1309–1312.

Laneuville P, Heisterkamp N and Groffen J. (1991). *Oncogene,* **6,** 275–282.

Leonard WJ and Lin JX. (2000). *J. Allergy Clin. Immunol.,* **105,** 877–888.

Leonard WJ and O'Shea JJ. (1998). *Annu. Rev. Immunol.,* **16,** 293–322.

Leonard WJ. (1996). *Annu. Rev. Med.,* **47,** 229–239.

Leverrier Y, Thomas J, Perkins GR, Mangeney M, Collins MK and Marvel J. (1997). *Oncogene,* **14,** 425–430.

Lim CP and Cao X. (2001). *J. Biol. Chem.,* **276,** 21004–21011.

Lin EY, Orlofsky A, Wang HG, Reed JC and Prystowsky MB. (1996). *Blood,* **87,** 983–992.

Lioubin MN, Algate PA, Tsai S, Carlberg K, Aebersold A and Rohrschneider LR. (1996). *Genes Dev.,* **10,** 1084–1095.

Liu KD, Gaffen SL, Goldsmith MA and Greene WC. (1997). *Curr. Biol.,* **7,** 817–826.

Lord KA, Abdollahi A, Thomas SM, DeMarco M, Brugge JS, Hoffman-Liebermann B and Liebermann DA. (1991). *Mol. Cell. Biol.,* **11,** 4371–4379.

Luo H, Hanratty WP and Dearolf CR. (1995). *EMBO J.,* **14,** 1412–1420.

Luo H, Rose P, Barber D, Hanratty WP, Lee S, Roberts TM, D'Andrea AD and Dearolf CR. (1997). *Mol. Cell. Biol.,* **17,** 1562–1571.

Lutticken C, Wegenka UM, Yuan J, Buschmann J, Schindler C, Ziemiecki A, Harpur AG, Wilks AF, Yasukawa K, Taga T, Kishimoto T, Barbieri G, Pellegrini S, Sendtner M, Heinrich P and Horn F. (1994). *Science,* **263,** 89–92.

Macchi P, Villa A, Giliani S, Sacco MG, Frattini A, Porta F, Ugazio AG, Johnston JA, Candotti F, O'Shea JJ, Vezzoni P and Notarangelo LD. (1995). *Nature,* **377,** 65–68.

Mathey-Prevot B, Nabel G, Palacios R and Baltimore D. (1986). *Mol. Cell. Biol.,* **6,** 4133–4135.

Matsuguchi T, Zhao Y, Lilly MB and Kraft AS. (1997). *J. Biol. Chem.,* **272,** 17450–17459.

Matsumoto A, Masuhara M, Mitsui K, Yokouchi M, Ohtsubo M, Misawa H, Miyajima A and Yoshimura A. (1997). *Blood,* **89,** 3148–3154.

McLemore ML, Grewal S, Liu F, Archambault A, Poursine-Laurent J, Haug J and Link DC. (2001). *Immunity* **14,** 193–204.

Metcalf D. (1989). *Nature,* **339,** 27–30.

Migliaccio G, Migliaccio AR, Kreider BL, Rovera G and Adamson JW. (1989). *J. Cell. Biol.,* **109,** 833–841.

Migone TS, Cacalano NA, Taylor N, Yi T, Waldmann TA and Johnston JA. (1998). *Proc. Natl. Acad. Sci. USA,* **95,** 3845–3850.

Miura O, Nakamura N, Quelle FW, Witthuhn BA, Ihle JN and Aoki N. (1994). *Blood,* **84,** 1501–1507.

Miyajima A, Kitamura T, Harada N, Yokota T and Arai K. (1992). *Annu. Rev. Immunol.,* **10,** 295–331.

Mui AL, Wakao H, Harada N, O'Farrell AM and Miyajima A. (1995a). *J. Leukoc. Biol.,* **57,** 799–803.

Mui AL, Wakao H, O'Farrell AM, Harada N and Miyajima A. (1995b). *EMBO J.,* **14,** 1166–1175.

Murata Y, Takaki S, Migita M, Kikuchi Y, Tominaga A and Takatsu K. (1992). *J. Exp. Med.,* **175,** 341–351.

Myers MP, Andersen JN, Cheng A, Tremblay ML, Horvath CM, Parisien JP, Salmeen A, Barford D and Tonks NK. (2001). *J. Biol. Chem.,* **276,** 47771–47774.

Nagata Y, Moriguchi T, Nishida E and Todokoro K. (1997a). *Blood,* **90,** 929–934.

Nagata Y, Nishida E and Todokoro K. (1997b). *Blood,* **89,** 2664–2669.

Naka T, Fujimoto M and Kishimoto T. (1999). *Trends Biochem. Sci.,* **24,** 394–398.

Naka T, Narazaki M, Hirata M, Matsumoto T, Minamoto S, Aono A, Nishimoto N, Kajita T, Taga T, Yoshizaki K, Akira S and Kishimoto T. (1997). *Nature,* **387,** 924–929.

Narazaki M, Fujimoto M, Matsumoto T, Morita Y, Saito H, Kajita T, Yoshizaki K, Naka T and Kishimoto T. (1998). *Proc. Natl. Acad. Sci. USA,* **95,** 13130–13134.

Narazaki M, Witthuhn BA, Yoshida K, Silvennoinen O, Yasukawa K, Ihle JN, Kishimoto T and Taga T. (1994). *Proc. Natl. Acad. Sci. USA,* **91,** 2285–2289.

Nelson BH, Lord JD and Greenberg PD. (1996). *Mol. Cell. Biol.,* **16,** 309–317.

Neubauer H, Cumano A, Muller M, Wu H, Huffstadt U and Pfeffer K. (1998). *Cell,* **93,** 397–409.

Nicholson SE and Hilton DJ. (1998). *J. Leukoc. Biol.,* **63,** 665–668.

Nicholson SE, Willson TA, Farley A, Starr R, Zhang JG, Baca M, Alexander WS, Metcalf D, Hilton DJ and Nicola NA. (1999). *EMBO J.,* **18,** 375–385.

Nicola NA, Robb L, Metcalf D, Cary D, Drinkwater CC and Begley CG. (1996). *Blood,* **87,** 2665–2674.

Nieborowska-Skorska M, Wasik MA, Slupianek A, Salomoni P, Kitamura T, Calabretta B and Skorski T. (1999). *J. Exp. Med.,* **189,** 1229–1242.

Noguchi M, Yi H, Rosenblatt HM, Filipovich AH, Adelstein S, Modi WS, McBride OW and Leonard WJ. (1993). *Cell,* **73,** 147–157.

Nosaka T, van Deursen JM, Tripp RA, Thierfelder WE, Witthuhn BA, McMickle AP, Doherty PC, Grosveld GC and Ihle JN. (1995). *Science,* **270,** 800–802.

Odai H, Sasaki K, Iwamatsu A, Hanazono Y, Tanaka T, Mitani K, Yazaki Y and Hirai H. (1995). *J. Biol. Chem.,* **270,** 10800–10805.

Okuda K, Sanghera JS, Pelech SL, Kanakura Y, Hallek M, Griffin JD and Druker BJ. (1992). *Blood,* **79,** 2880–2887.

Okutani Y, Kitanaka A, Tanaka T, Kamano H, Ohnishi H, Kubota Y, Ishida T and Takahara J. (2001). *Oncogene,* **20,** 6643–6650.

O'Shea JJ, Notarangelo LD, Johnston JA and Candotti F. (1997). *J. Clin. Immunol.,* **17,** 431–447.

Packham G, White EL, Eischen CM, Yang H, Parganas E, Ihle JN, Grillot DA, Zambetti GP, Nunez G and Cleveland JL. (1998). *Genes Dev.,* **12,** 2475–2487.

Pallen CJ, Tan YH and Guy GR. (1992). *Curr. Opin. Cell. Biol.,* **4,** 1000–1007.

Parganas E, Wang D, Stravopodis D, Topham DJ, Marine JC, Teglund S, Vanin EF, Bodner S, Colamonici OR, van Deursen JM, Grosveld G and Ihle JN. (1998). *Cell,* **93,** 385–395.

Park SY, Saijo K, Takahashi T, Osawa M, Arase H, Hirayama N, Miyake K, Nakauchi H, Shirasawa T and Saito T. (1995). *Immunity,* **3,** 771–782.

Paulson RF and Bernstein A. (1995). *Semin. Immunol.,* **7,** 267–277.

Pawson T. (1995). *Nature,* **373,** 573–580.

Pazdrak K, Adachi T and Alam R. (1997). *J. Exp. Med.,* **186,** 561–568.

Pazdrak K, Stafford S and Alam R. (1995). *J. Immunol.,* **155,** 397–402.

Peeters P, Raynaud SD, Cools J, Wlodarska I, Grosgeorge J, Philip P, Monpoux F, Van Rompaey L, Baens M, Van den Berghe H and Marynen P. (1997). *Blood,* **90,** 2535–2540.
Pelicci G, Lanfrancone L, Grignani F, McGlade J, Cavallo F, Forni G, Nicoletti I, Pawson T and Pelicci PG. (1992). *Cell,* **70,** 93–104.
Pellegrini S and Dusanter-Fourt I. (1997). *Eur. J. Biochem.,* **15,** 615–633.
Pellegrini M and Strasser A. (1999). *J. Clin. Immunol.,* **19,** 365–377.
Perkins GR, Marshall CJ and Collins MK. (1996). *Blood,* **87,** 3669–3675.
Pierce J, Potter M, Scott A, Humphries J, Aaronson SA and Ihle JN. (1985). *Cell,* **41,** 685–692.
Pierce JH. (1989). *Biochim. Biophys. Acta,* **989,** 179–208.
Pratt JC, Weiss M, Sieff CA, Shoelson SE, Burakoff SJ and Ravichandran KS. (1996). *J. Biol. Chem.,* **271,** 12137–12140.
Quelle FW, Sato N, Witthuhn BA, Inhorn RC, Eder M, Miyajima A, Griffin JD and Ihle JN. (1994). *Mol. Cell. Biol.,* **14,** 4335–4341.
Quelle FW, Thierfelder W, Witthuhn BA, Tang B, Cohen S and Ihle JN. (1995). *J. Biol. Chem.,* **270,** 20775–20780.
Quelle FW, Wang J, Feng J, Wang D, Cleveland JL, Ihle JN and Zambetti GP. (1998). *Genes Dev.,* **12,** 1099–1107.
Raines MA, Golde DW, Daeipour M and Nel AE. (1992). *Blood,* **79,** 3350–3354.
Rao P and Mufson RA. (1995). *J. Biol. Chem.,* **270,** 6886–6893.
Reddy EP, Smith MJ and Srinivasan A. (1983). *Proc. Natl. Acad. Sci. USA,* **80,** 3623–3627.
Reddy EP, Korapati A, Chaturvedi P and Rane S. (2000). *Oncogene,* **19,** 2532–2547.
Reed JC. (1997). *Nature,* **387,** 773–776.
Rinaudo MS, Su K, Falk LA, Halder S and Mufson RA. (1995). *Blood,* **86,** 80–88.
Rodig SJ, Meraz MA, White JM, Lampe PA, Riley JK, Arthur CD, King KL, Sheehan KC, Yin L, Pennica D, Johnson Jr EM and Schreiber RD. (1998). *Cell,* **93,** 373–383.
Rovera G, Valtieri M, Mavilio F and Reddy EP. (1987). *Oncogene,* **1,** 29–35.
Rozakis-Adcock M, Fernley R, Wade J, Pawson T and Bowtell D. (1993). *Nature,* **363,** 83–85.
Russell SM, Johnston JA, Noguchi M, Kawamura M, Bacon CM, Friedmann M, Berg M, McVicar DW, Witthuhn BA, Silvennoinen O, Goldman AS, Schmalstieg FC, Ihle JN, O'Shea JJ and Leonard WJ. (1994). *Science,* **266,** 1042–1045.
Russell SM, Tayebi N, Nakajima H, Riedy MC, Roberts JL, Aman MJ, Migone TS, Noguchi M, Markert ML, Buckley RH, O'Shea JJ and Leonard WJ. (1995). *Science,* **270,** 797–800.
Saharinen P, Takaluoma K and Silvennoinen O. (2000). *Mol. Cell. Biol.,* **20,** 3387–3395.
Sakai I and Kraft AS. (1997). *J. Biol. Chem.,* **272,** 12350–12358.
Sasaki A, Yasukawa H, Shouda T, Kitamura T, Dikic I and Yoshimura A. (2000). *J. Biol. Chem.,* **275,** 29338–29347.
Sato N, Sakamaki K, Terada N, Arai K and Miyajima A. (1993). *EMBO J.,* **12,** 4181–4189.
Sato S, Katagiri T, Takaki S, Kikuchi Y, Hitoshi Y, Yonehara S, Tsukada S, Kitamura D, Watanabe T and Witte O. (1994). *J. Exp. Med.,* **180,** 2101–2111.
Satoh T, Nakafuku M, Miyajima A and Kaziro Y. (1991). *Proc. Natl. Acad. Sci. USA,* **88,** 3314–3318.
Sattler M, Salgia R, Okuda K, Uemura N, Durstin MA, Pisick E, Xu G, Li JL, Prasad KV and Griffin JD. (1996). *Oncogene,* **12,** 839–846.
Schaefer TS, Sanders LK and Nathans D. (1995). *Proc. Natl. Acad. Sci. USA,* **92,** 9097–9101.
Schindler C and Darnell Jr JE. (1995). *Annu. Rev. Biochem.,* **64,** 621–651.
Schindler C. (1999). *Exp. Cell. Res.,* **253,** 7–14.
Selvakumaran M, Liebermann DA and Hoffman-Liebermann B. (1992). *Mol. Cell. Biol.,* **12,** 2493–2500.
Silvennoinen O, Ihle JN, Schlessinger J and Levy DE. (1993a). *Nature,* **366,** 583–585.
Silvennoinen O, Witthuhn BA, Quelle FW, Cleveland JL, Yi T and Ihle JN. (1993b). *Proc. Natl. Acad. Sci. USA,* **90,** 8429–8433.
Smith A, Metcalf D and Nicola NA. (1997). *EMBO J.,* **16,** 451–464.
Songyang Z, Baltimore D, Cantley LC, Kaplan DR and Franke TF. (1997). *Proc. Natl. Acad. Sci. USA,* **94,** 11345–11350.
Stahl N and Yancopoulos GD. (1993). *Cell,* **74,** 587–590.
Starr R, Willson TA, Viney EM, Murray LJ, Rayner JR, Jenkins BJ, Gonda TJ, Alexander WS, Metcalf D, Nicola NA and Hilton DJ. (1997). *Nature,* **387,** 917–921.
Strous GJ, van Kerkhof P, Govers R, Ciechanover A and Schwartz AL. (1996). *EMBO J.,* **15,** 3806–3812.
Takaki S and Takatsu K. (1994). *Int. Arch. Allergy Immunol.,* **104,** 36–38.
Takaki S, Kanazawa H, Shiiba M and Takatsu K. (1994). *Mol. Cell. Biol.,* **14,** 7404–7413.
Taniguchi T. (1995). *Science,* **268,** 251–255.
Tanner JW, Chen W, Young RL, Longmore GD and Shaw AS. (1995). *J. Biol. Chem.,* **270,** 6523–6530.
Tanuma N, Nakamura K, Shima H and Kikuchi K. (2000). *J. Biol. Chem.,* **275,** 28216–28221.
Terada K, Kaziro Y and Satoh T. (1995). *J. Biol. Chem.,* **270,** 27880–27886.
Terada K, Kaziro Y and Satoh T. (1997). *J. Biol. Chem.,* **272,** 4544–4548.
Thomis DC, Gurniak CB, Tivol E, Sharpe AH and Berg LJ. (1995). *Science,* **270,** 794–797.
Tilton B, Andjelkovic M, Didichenko SA, Hemmings BA and Thelen M. (1997). *J. Biol. Chem.,* **272,** 28096–28101.
Torigoe T, O'Connor R, Santoli D and Reed JC. (1992). *Blood,* **80,** 617–624.
Turkson J, Ryan D, Kim JS, Zhang Y, Chen Z, Haura E, Laudano A, Sebti S, Hamilton AD and Jove R. (2001). *J. Biol. Chem.,* **276,** 45443–45455.
Uchida K, Yoshimura A, Inazawa J, Yanagisawa K, Osada H, Masuda A, Saito T, Takahashi T and Miyajima A. (1997). *Cytogenet. Cell. Genet.,* **78,** 209–212.
Valtieri M, Tweardy DJ, Caracciolo D, Johnson K, Mavilio F, Altmann S, Santoli D and Rovera G. (1987). *J. Immunol.,* **138,** 3829–3835.
van Dijk TB, Caldenhoven E, Raaijmakers JA, Lammers JW, Koenderman L and de Groot RP. (1997). *FEBS Lett.,* **412,** 161–164.
VanderKuur JA, Wang X, Zhang L, Campbell GS, Allevato G, Billestrup N, Norstedt G and Carter-Su C. (1994). *J. Biol. Chem.,* **269,** 21709–21717.
Verdier F, Chretien S, Muller O, Varlet P, Yoshimura A, Gisselbrecht S, Lacombe C and Mayeux P. (1998). *J. Biol. Chem.,* **273,** 28185–28190.
Vinkemeier U, Moarefi I, Darnell JE and Kuriyan J. (1998). *Science,* **279,** 1048–1052.

Wang Y, Morella KK, Ripperger J, Lai CF, Gearing DP, Fey GH, Campos SP and Baumann H. (1995). *Blood,* **86,** 1671–1679.

Ward AC, Touw I and Yoshimura A. (2000). *Blood,* **95,** 19–29.

Watowich SS, Hilton DJ and Lodish HF. (1994). *Mol. Cell. Biol.,* **14,** 3535–3549.

Weiss A and Schlessinger J. (1998). *Cell,* **94,** 277–280.

Welham MJ, Duronio V, Sanghera JS, Pelech SL and Schrader JW. (1992). *J. Immunol.,* **149,** 1683–1693.

Wen Z, Zhong Z and Darnell Jr JE. (1995). *Cell,* **82,** 241–250.

Witthuhn BA, Quelle FW, Silvennoinen O, Yi T, Tang B, Miura O and Ihle JN. (1993). *Cell,* **74,** 227–236.

Woodcock JM, Bagley CJ and Lopez AF. (1999). *Int. J. Biochem. Cell. Biol.,* **31,** 1017–1025.

Woodcock JM, Bagley CJ, Zacharakis B and Lopez AF. (1996). *J. Biol. Chem.,* **271,** 25999–26006.

Xu X, Sun YL and Hoey T. (1996). *Science,* **272,** 794–797.

Yamamoto K, Quelle FW, Thierfelder WE, Kreider BL, Gilbert DJ, Jenkins NA, Copeland NG, Silvennoinen O and Ihle JN. (1994). *Mol. Cell. Biol.,* **14,** 4342–4349.

Yasukawa H, Misawa H, Sakamoto H, Masuhara M, Sasaki A, Wakioka T, Ohtsuka S, Imaizumi T, Matsuda T, Ihle JN and Yoshimura A. (1999). *EMBO J.,* **18,** 1309–1320.

Yasukawa H, Sasaki A and Yoshimura A. (2000). *Annu. Rev. Immunol.,* **18,** 143–164.

Yetter D. (1995). *Nurs. Manage.,* **26,** 60.

Yin T, Shen R, Feng GS and Yang YC. (1997). *J. Biol. Chem.,* **272,** 1032–1037.

Yoshimura A. (1998a). *Cytokine Growth Factor Rev.,* **9,** 197–204.

Yoshimura A. (1998b). *Leukemia,* **12,** 1851–1857.

Yoshimura A, Ohkubo T, Kiguchi T, Jenkins NA, Gilbert DJ, Copeland NG, Hara T and Miyajima A. (1995). *EMBO J.,* **14,** 2816–2826.

You M, Yu DH and Feng GS. (1999). *Mol. Cell. Biol.,* **19,** 2416–2424.

Yu CL and Burakoff SJ. (1997). *J. Biol. Chem.,* **272,** 14017–14020.

Yu CL, Meyer DJ, Campbell GS, Larner AC, Carter-Su C, Schwartz J and Jove R. (1995). *Science,* **269,** 81–83.

Zhang JG, Farley A, Nicholson SE, Willson TA, Zugaro LM, Simpson RJ, Moritz RL, Cary D, Richardson R, Hausmann G, Kile BJ, Kent SB, Alexander WS, Metcalf D, Hilton DJ, Nicola NA and Baca M. (1999). *Proc. Natl. Acad. Sci. USA,* **96,** 2071–2076.

Zhang Z, Blenis J, Li H, Schindler C and Chen-Kiang S. (1995). *Science.* **267,** 1990–1994.

Zhao Y, Wagner F, Frank SJ and Kraft AS. (1995). *J. Biol. Chem.,* **270,** 13814–13818.

Zhou YJ, Hanson EP, Chen YQ, Magnuson K, Chen M, Swann PG, Wange RL, Changelian PS and O'Shea JJ. (1997). *Proc. Natl. Acad. Sci. USA,* **94,** 13850–13855.

Zon LI. (1995). *Blood,* **86,** 2876–2891.