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Cytokine Signaling in 2002: New Surprises in the Jak/Stat Pathway

Review

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The importance of Jak-Stat pathway signaling in regulating cytokine-dependent gene expression and cellular development/survival is well established. Nevertheless, advances continue to be made in defining Jak-Stat pathway effects on different cellular processes and in different organisms. This review focuses on recent advances in the field and highlights some of the most active areas of Jak-Stat pathway research.

Introduction

Cytokines that bind the Type I family of cytokine receptors play critical roles in the development and differentiation of diverse cells. Like the interferons (IFNs), which bind receptors designated as the Type II cytokine receptor family, Type I cytokines also have essential functions in host defense. Investigation of how these extracellular messengers regulate cell function led to the elucidation of the roles of Janus kinases and the Stat (signal transducer and activation of transcription) family of transcription factors, establishing this as a remarkably simple pathway for membrane to nucleus signaling. In this review the most recent advances in this field will be emphasized, but even this is not a simple task, as within the last 2 years alone more than 1500 articles have been published on Jaks and Stats. Our purpose then is not to provide a comprehensive, historical review but rather to discuss the major lessons learned, examine the remaining controversies, and comment on new developments. References from the last century (including our own) will not be cited; interested readers are directed to the following reviews: Darnell et al. (1994); Ihle (1995); and Leonard and O'Shea (1998).

New Players on the Field—Recently Identified Cytokines and Receptors

New cytokines and receptors are continuing to be identified, and it is likely that there are still a number yet to be characterized. Interleukin (IL)-21 is a new member of the cytokine subfamily that binds the common γ chain, γc, and is produced by activated T cells (Asao et al., 2001; Parrish-Novak et al., 2000). Thymic stromal lymphopoietin is another cytokine that binds the IL-7α chain, but apparently does not require γc for signaling (Pandey et al., 2000; Park et al., 2000b). IL-12 is the product of two genes, which encode subunits designated p35 and p40, the latter having homology to cytokine receptors. Recently a new partner for p40 has been identified, p19. This new cytokine is denoted IL-23 (Oppmann et al., 2000), and it binds IL-12Rβ1 but not IL-12Rβ2. The receptor WSX-1/TCCR has homology to IL-12Rβ2, and its deficiency is associated with deficits in cell-mediated immune responses and IFN-γ production (Chen et al., 2000b; Yoshida et al., 2001); this phenotype makes it a possible candidate for an IL-23R subunit.

The IFN family or Type II family of cytokine receptors includes the receptors for IFN-α/β, IFN-γ, IFN-ω, and IL-10. New members of this family include limitin, IL-19, IL-20, IL-22/ IL-TIF, IL-24 (mda-7), and IL-26 (AK155) (Blumberg et al., 2001; Takahashi et al., 2001; Xie et al., 2000). The precise physiologic functions of these new cytokines are uncertain, but this will surely be of interest.

Efforts are now being made to create artificial, nonprotein ligands that bind Type I cytokine receptors based on the crystal structure of the ligand/receptor interactions (Guo et al., 2000). Given the wide physiologic functions of this family, it is exciting to consider the possibility that small molecule agonists/antagonists will be generated.

Janus Kinases

With completion of the draft sequence of the human genome, it appears that only four members of the mammalian Janus kinase family exist: Jak1, Jak2, Jak3, and Tyk2 (Figure 1). Large kinases of approximately one thousand amino acids, the Jaks have clear nonredundant in vivo functions, identified by the analysis of mice, and in the case of Jak3, humans deficient in this kinase.

In Vivo Function

Jak3

Jak3 selectively associates with γc and not other cytokine receptors. Accordingly, mutation of γc or Jak3 results in severe combined immunodeficiency, characterized by the lack of T and NK cells, but not B cells, thus designated T^-B^+ SCID (reviewed in Notarangelo et al., [2001]). Gene targeting of Jak3 in mice also results in SCID, with reduced numbers of lymphoid cells and a near absence of thymic progenitor cells (Baird et al., 2000). This is likely attributable to the failure of IL-7 signaling, whereas the absence of NK cell development has been attributed to the impairment in IL-15 signaling. The lymphoid cells produced have a high apoptotic index, and accordingly Jak3 has been found to be a key regulator of Bcl-2 and Bax (Wen et al., 2001).

A second important form of apoptosis in lymphocytes is activation-induced cell death (AICD). Regulated by cytokines, this form of apoptosis is not Bcl-2 dependent but is, rather, controlled by engagement of Fas. Notably, $Jak3^{-/-}$ mice have expansion of $CD4^+$ T cells, which express activation markers but have a restricted TCR repertoire (Gozalo-Sanmillan et al., 2001). This is consistent with the importance of IL-2 in regulating peripheral tolerance and lymphoid homeostasis. Thus, Jak3-deficient lymphocytes exhibit the paradox of having both too much and too little apoptosis. Recently, a Jak3 SCID family was identified with apparently leaky mutations

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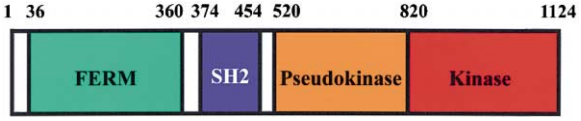

Figure 1. Schematic of Janus Kinase Structure

Janus kinases comprise FERM, SH2, pseudokinase, and kinase domains. The FERM domain mediates receptor interactions. Both the FERM and pseudokinase domains regulate catalytic activity.

that allowed for T cell development. This was associated with severe lymphoproliferative disease in one child, presumably due to defective AICD (Frucht et al., 2001b).

Jak1

In contrast to Jak3, Jak1 is widely expressed and associates with the IFN receptors and receptors that use gp130 and γc. Jak1$^{-/-}$ mice have grossly normal nonlymphoid organogenesis; however, Jak1$^{-/-}$ mice die perinatally of an ill-characterized defect that may be neurologic. Presumably this is due to failure of signaling via cytokines that use gp130, which require Jak1 and promote neuronal survival. Like Jak3$^{-/-}$ mice, Jak1$^{-/-}$ mice have SCID, consistent with the idea that Jak1 binds to the ligand-specific receptor subunit of γc-using cytokines. Using cells from these mice, Jak1 also was found to be essential for signaling by IFN-α/β and IFN-γ, in agreement with prior studies using a mutagenized cell line.

Jak2

Like Jak1, Jak2 is widely expressed and is involved in signaling by single chain hormone receptors, the common β chain family, and certain members of the class II receptor cytokine family. Targeting of the murine Jak2 gene resulted in embryonic lethality at day 12.5 due to failure of erythropoiesis. Cells from these mice showed that Jak2 is essential for IL-3, granulocyte macrophage colony stimulating factor (GM-CSF), IL-5, thrombopoietin (Tpo), and IFN-γ, but not IL-6 and IFNα/β signaling. Transfer of Jak2$^{-/-}$ fetal liver cells into irradiated Jak3$^{-/-}$ recipients resulted in normal thymic subsets, arguing that Jak2 is not essential for T cell development.

A chromosomal translocation, [t(9;12)(p24;p13)], occurs in a subset of leukemias, creating a fusion protein comprising the dimerization domain of the transcription factor Tel and the kinase domain (or, in some cases, pseudokinase and kinase domains) of Jak2. Other translocations may result in Tel-Jak3 and Tel-Tyk2 fusion proteins.

Tyk2

First identified as an essential component in a screen for mutants in IFN-α signaling, Tyk2$^{-/-}$ mice have remarkably subtle defects in IFN-α/β signaling (Karaghiosoff et al., 2000; Shimoda et al., 2000), perhaps one of the biggest surprises in the field. Cells from these mice are unresponsive to low doses of type I IFNs but have relatively normal antiviral responses. IL-10 signaling is essentially normal and IL-12 responses were reduced, but not absent. Thus, unlike other Jaks, the in vivo nonredundant functions of Tyk2 are modest.

Structure

Presently, there is no detailed structural information on this class of protein tyrosine kinases despite several unique features. One such feature is the pseudokinase domain, which has essential regulatory functions (Chen et al., 2000a; Saharinen et al., 2000; Yeh et al., 2000). A number of patients with Jak3-SCID have mutations in the pseudokinase domain. Conversely, a mutation of in the JH2 domain of the *Drosophila* Jak, Hopscotch, is transforming (Zeidler et al., 2000); however, without actual structural data, one can only speculate as to how these mutants might be exerting their effects.

The amino terminus of the Jaks is a relatively divergent region of about 300 amino acids that has homology to band four point one, ezrin, radixin, and moesin and is referred to as the FERM domain. The Jak FERM domain mediates receptor association and regulates catalytic activity (Hilkens et al., 2001; Zhou et al., 2001).

For some receptors, including the IFNα/βR, EpoR, PrlR, and oncostatin M (OSM)R, the Jak FERM domain provides a chaperone function, influencing plasma membrane expression of the receptor (Huang et al., 2001; Radtke et al., 2002). However, this may not be requisite for all cytokine receptors. For instance, in the absence of Jak3, γc expression is evidently not impaired (Suzuki et al., 2000). Clearly, these are interesting findings that warrant further study.

Jak Associated Proteins

In addition to cytokine receptors, the SH3- and ITAM-containing molecule STAM1 also associates with Jak2 and Jak3, is phosphorylated in response to cytokines, and augments signaling. However, Stam1$^{-/-}$ mice have normal hematopoietic development and cytokine signaling (Yamada et al., 2001). The relatively mild phenotype associated with STAM1 deficiency may be related to the existence of a second STAM protein, STAM2 (Endo et al., 2000) (Pandey et al., 2000). Additionally, the SH2 containing proteins SH2Bβ and Aps bind and regulate the catalytic activity of some but not all Jaks (O'Brien et al., 2001). Thus, there appear to be a variety of proteins that can regulate Jaks; but despite the overall structural conservation of these kinases, they may be regulated in subtly different manners.

In summary, it is clear that many questions remain pertaining to the function and regulation of Janus kinases. However, despite the gaps in our knowledge, there is general agreement on the overall scheme of cytokine signaling (Figure 2): ligand binding to cytokine receptors results in the activation of prebound Jak kinases, which auto- and transphosphorylate and phosphorylate the cytokine receptor on tyrosine residues. The phosphorylated receptor is then recognized by proteins with SH2 or PTB domains, which themselves become phosphorylated. One class of SH2 containing proteins, the Stats, latent cytosolic transcription factors, is particularly important in explaining cytokine action.

Stats

Stat1 and Stat2 were first identified as complexes that bound to response elements of interferon-inducible genes. Now, a total of seven mammalian Stats have been identified: Stat1, Stat2, Stat3, Stat4, Stat5a, Stat5b, and Stat6 (Figure 3); again, large-scale sequencing efforts have failed to identify new members of this family (Horvath, 2000; Ihle, 2001). It is now clear that this small family has critical functions in cytokine signal but is also activated by other receptors, as well.

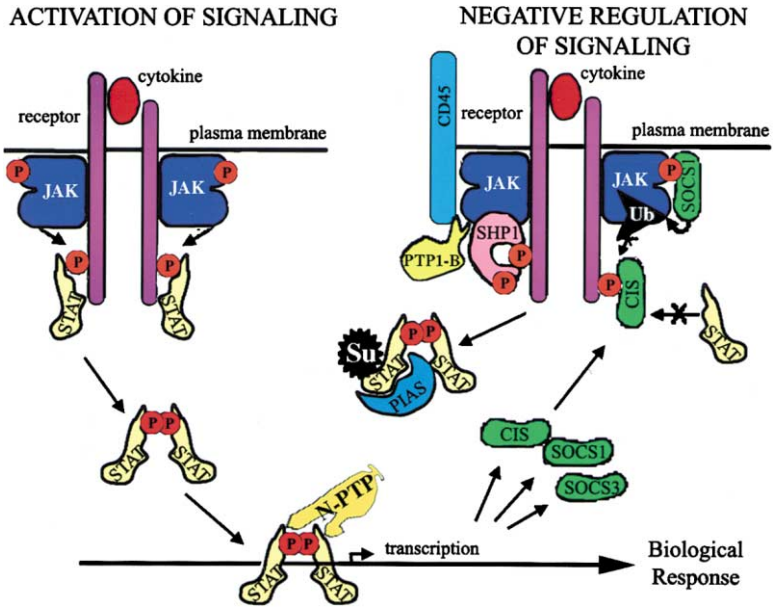

Figure 2. Overview of Cytokine Signaling: Positive and Negative Regulation

Cytokines bind to homodimeric or heterodimeric receptors, which bind Janus kinases (Jaks). Jaks are activated by transphorylation and they in turn phosphorylate cytokine receptors, allowing Stats to bind via SH2-phosphotyrosine interactions. Stats themselves are phosphorylated, permitting Stat dimerization and translocation to the nucleus where Stats bind DNA and regulate gene expression. This process is regulated at multiple steps, some of which are summarized here: tyrosine phosphatases such as SHP-1, CD45, and PTP1b may regulate phosphorylation of receptors and Jaks. Dimerized Stats can be bound by PIAS members, which have been found to be SUMO E3 ligases; the depicted sumoylation of Stats, however, is a speculative, albeit reasonable, possibility. Additionally, cytokine stimulation induces the transcription of a family of SH2 containing proteins known as SOCS proteins. SOCS proteins inhibit signaling by multiple means: (1) binding and inhibiting Jaks, (2) binding cytokine receptors and blocking Stats recruitment, and (3) promoting ubiquitination and degradation of the Jak/receptor complex. Stats are dephosphorylated in the nucleus, but the identity of the predominant nuclear Stat phosphatase (N-PTP) remains to be determined.

Stat Structure and Function

Stats are proteins of 750 to 850 amino acids that contain the following domains: amino terminal, coiled-coil, SH2, linker, DNA binding, and transcriptional activation domains. The crystal structure of two Stats, Stat1 and Stat3, bound to DNA has been solved (reviewed in (Horvath, 2000), but the structures did not include the amino- and carboxy-terminal portions of the molecules. The SH2 domain is an essential feature for Stat activation and function, docking the protein to tyrosine phosphorylated receptor subunits. Additionally though, Stat2 and perhaps also Stat1 associate with the unactivated, nonphosphorylated IFNAR prior to ligand binding. The receptor-bound Stats are then phosphorylated by Jaks on a conserved tyrosine residue and the SH2 domains mediate dimerization through reciprocal phosphotyrosine/SH2 interactions. The Stat SH2 domain may also be important for association with the activating Jak.

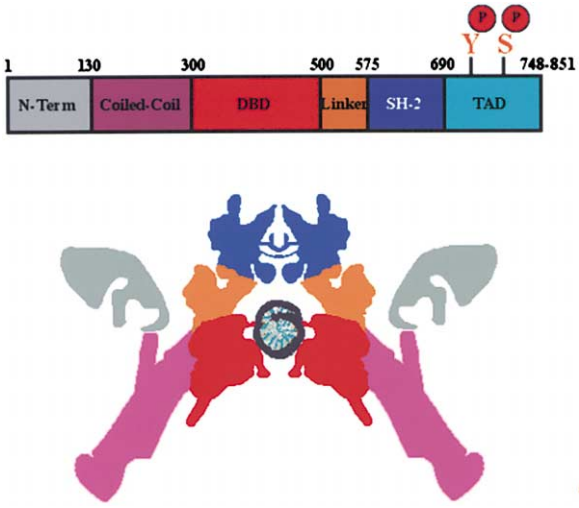

Figure 3. Structure of Stats and the Phenotype of Stat Knockout Mice

Stat proteins have amino terminal, coiled-coil, DNA binding, linker, SH2, and transcriptional activation domains. The structure of a polypeptide lacking the amino terminal domain and the TAD has been solved and is shown. Though the structure of the amino terminal domain in isolation has been determined, the placement shown here is conjectural.

The phosphorylated Stat dimer bound to DNA forms a nutcracker-like structure with the SH2 domains forming the hinge (Figure 3). The central portion (approximately aa 320-480) of the dimer forms a β barrel with an immunoglobulin fold similar to NF-κB and p53 and is responsible for DNA binding, although the residues that directly contact DNA are limited. Stat homodimers bind a motif termed a GAS (γ activated sequence) element ($TTN_{5-6}AA$). Unlike other cytokines, IFN-α/β induces the formation of a complex comprising Stat1, Stat2, and IRF9 that binds the IFN-α/β-stimulated response element (ISRE), $AGTTN_3TTTC$.

While the importance of tyrosine phosphorylation and dimerization of Stat proteins is clear, the mechanisms that regulate nuclear import and export are an area of intense investigation. Accumulation of Stat proteins in the nucleus is clearly controlled by nuclear export via Ran-dependent interaction with chromosome region maintenance (CRM)1/exportin 1 (McBride et al., 2000). A Leu-rich motif in the DNA binding domain of Stat1 (amino acids 400-409), conserved in other Stats, is critical for nuclear export. DNA binding of the phosphorylated Stat dimer is proposed to mask the NES, whereas Stat dephosphorylation and dissociation from DNA permits recognition of this site, allowing nuclear export. It should be noted that a different NES (amino acids 302-314) has also been mapped (Begitt et al., 2000).

Inhibition of nuclear export alone is not sufficient to

cause Stat nuclear accumulation; Stats are imported by the nuclear import receptor, importin-α5, and Ran. This requires Stat dimerization, and tyrosine phosphorylation alone is not sufficient (McBride et al., 2002). Stat1 L407 appears to function as nuclear localization signal (NLS), as mutations in this region of the protein interfere with importin binding and nuclear import of phosphorylated Stat dimers (Melen et al., 2001; Meyer et al., 2002; McBride et al. 2002). However, a bonafide, autonomously functioning Stat NLS has yet to be defined. Additionally, nuclear trafficking of nonphosphorylated Stat monomers may occur by mechanisms distinct from those that govern movement of dimers (Lillemeier et al., 2001; Meyer et al., 2002).

Stat Transcriptional Regulation

The Stat C terminus contains an autonomously functioning transcriptional activation domain (TAD), and alternatively spliced isoforms of Stat1, Stat3, and Stat4 lacking this domain have attenuated transcriptional activity. Truncated Stat5 polypeptides lacking the TAD are thought to be generated by proteolysis and are unstable (Wang et al., 2000). While the mechanisms by which these domains regulate transcription are incompletely defined, serine phosphorylation within the TAD typically promotes transcriptional activity and enhances the expression of selected genes (Decker and Kovarik, 2000; Kovarik et al., 2001). This site, a putative mitogen-activated protein kinase phosphorylation motif, encompasses serine 727 in Stat1 and Stat3. Stat4, Stat5a, and Stat5b have similar residues in analogous positions, but the identity of the kinase(s) responsible for these modifications remains a subject of some controversy. Nonetheless, TAD phosphorylation could allow for regulation and crosstalk by different receptors. The mechanism underlying this regulation presumably involves the recruitment of other transcription factors and coactivators. Factors shown to bind the TAD include CBP/p300, c-Jun, MCM5, and BRCA1 (Horvath, 2000). CBP/p300 also binds other parts of the Stat molecule, and the N-myc interacting protein (Nmi-1) facilitates this association. Stat2, however, recruits a different histone acetyltransferase, GCN5 in complex with TAF130 (Paulson et al., 2002).

Stats also interact with a wide variety of other factors; in addition to p48/IRF9, these factors include NF-κB, SMADs, Sp1, USF-1, c-Jun, PU.1, C/EBPβ, glucocorticoid receptor, NcoA-1, YY-1, TFII-1, and HMG-I(Y). Some of these interactions are mediated by the Stat coiled-coil domain, but the Stat linker domain is also involved in transcriptional control. Additionally, the amino terminus of the Stats forms a hook-like domain, which may facilitate Stat interactions and cooperative binding to tandem imperfect Stat binding sites. Thus, despite what we have learned about Stats and transcriptional regulation, it is very clear that we are only beginning to understand these processes and the relevant interactions.

Biological Functions of Stats

Stat1

The importance of Stat1 in IFN-γ and IFNα/β signaling was clearly established by the generation of Stat1-deficient mice, confirming the initial findings in mutagenized cell lines (Darnell et al., 1994). Stat1$^{-/-}$ mice are highly susceptible to microbial and viral infections and tumor formation due to severely impaired IFN responses, with the induction of many well-known IFN-inducible genes being abrogated. Although a number of cytokines and growth factors can activate Stat1, to date there are no major developmental deficits identified in these mice attributable to non-IFN signaling. A missense mutation of Stat1 has been identified in a patient suffering from atypical mycobacterial infections, similar to individuals with mutations of IFNγR subunits. Interestingly though this Stat1 mutation was not associated with susceptibility to viral infections (Dupuis et al., 2001).

Naive CD4^{+} T cells differentiate to T helper (Th)1 cells, which produce IFN-γ and promote cell-mediated immunity or Th2 cells, which drive allergic and anti-helminthic responses. The transcription factor T-bet has recently been shown to be an important regulator of Th1 differentiation. IFN-γ induces expression of T-bet in a feed-forward mechanism, enhancing the ability of T cells to produce IFN-γ; this regulation is Stat1 dependent (Lighvani et al., 2001). However, T-bet expression is not completely absent in Stat1-deficient mice, arguing for Stat1-independent modes of T-bet regulation. Also, Stat1 activation is not sufficient to induce T-bet, since IFN-α/β did not induce expression of this transcription factor.

IFNs are typically growth inhibitory and promote immune recognition of target cells; thus, the demonstration that Stat1-deficient mice exhibit a higher incidence of spontaneous and chemically induced tumors was very provocative (Shankaran et al., 2001). These findings have generated renewed interest in the notion of immune surveillance, with IFN-γ signaling serving as a tumor suppressor system. The revised concept, however, is that tumors with impaired responsiveness to IFN-γ have a selective advantage, allowing survival in an immunocompetent host. There are likely multiple mechanisms that underlie this process. Stat1 appears to have important proapoptotic effects. Additionally, it controls the expression of proteins involved in antigen presentation, thus affecting the immunogenicity of the tumor.

It should be noted that not all IFN-γ signaling is abrogated in Stat1$^{-/-}$ cells. Using microarray technology and representational difference analysis, a surprisingly large number of Stat1-independent genes were found to be induced by IFN-γ, consistent with the observations that Stat1$^{-/-}$ mice are less susceptible to infection than mice lacking both IFN-γ and IFNα/β receptors (Gil et al., 2001; Ramana et al., 2001). Though IFNs typically inhibit growth, in the absence of Stat1, they can be growth promoting. Indeed, another class of genes can be identified–genes not ordinarily induced and even repressed by IFN-γ when Stat1 is present, but that are induced in its absence; examples include c-*jun*, c-*myc*, and IFN-γ (Nguyen et al., 2000). While the physiologic significance of this regulation remains to be determined, the levels of Stat1 are dynamic; one can therefore imagine complex scenarios of gene regulation that may depend upon the level of expression of Stat1. Of note, IFN-mediated suppression of B cell growth is Stat1 independent, but this appears to be due in part to the induction of the proapoptotic molecule DAXX (Gongora et al., 2001).

While on the whole the deficits in Stat1$^{-/-}$ mice are indicative of IFN deficiency, IFN-independent roles of Stat1 have been documented. For instance, natural killer

cell function is more severely impaired by Stat1 deficiency than absence of both IFN-γ and IFNα/β receptors (Lee et al., 2000).

In vivo, there is limited information supporting a critical role for Stat1 in growth factor signaling; however, there is one example in which Stat1 can play an interesting role in modulating signaling via a receptor tyrosine kinase. Activating mutations of fibroblast growth factor (FGF) receptors are associated with several forms of human dwarfism and craniosynostosis syndromes. Absence of Stat1 counteracts these effects, suggesting that it may be an important aspect of FGF signaling that leads to this pathology (Sahni et al., 2001). A consistent theme that emerges is that Stat1 serves as a negative regulator of cell growth. As will be apparent shortly, this contrasts sharply with the function of other Stats, especially Stat3 and Stat5.

Stat2

The major function of Stat2 is its role in IFN-α/β signaling, where it complexes with Stat1 and IRF9 to bind IRSEs. Accordingly, Stat2 knockout mice are fertile and viable but are susceptible to viral infections, having impaired IFN-α/β responsiveness (Park et al., 2000a). Stat1 upregulation in response of IFN-α is also impaired in Stat2-deficient cells. As a result, IFN-α induction of GAS-dependent genes, which are presumably not directly dependent upon Stat2, is impaired nonetheless. However, the lack of IFN-α responsiveness of GAS-driven genes is not apparent in Stat2$^{-/-}$ macrophages, which express normal levels of Stat1.

Stat3

Originally identified as an acute-phase response factor, activated by IL-6, Stat3 is also activated by many other cytokines. Targeted deletion of Stat3 is embryonically lethal at day 7.5, necessitating the production of tissue-specific knockouts using Cre-lox technology (Akira, 2000). In general, tissue-specific targeting of Stat3 has not been found to have major developmental consequences; however, other abnormalities are apparent. Stat3-deficient T cells and hepatocytes have poor responses to IL-6 (Alonzi et al., 2001), whereas Stat3 deficiency in macrophages and neutrophils is associated with exaggerated production of cytokines presumably due to impaired IL-10 responsiveness. Deletion of Stat3 in mammary glands results in delayed programmed cell death that occurs during cyclical mammary gland involution. Lack of Stat3 in keratinocytes permits normal initial development of skin and hair, but subsequent hair cycles are disrupted. Skin wound healing, in vitro migration of epidermal cells, and thymic architecture are also disrupted (Sano et al., 2001).

Very recently, selective targeting of the Stat3b isoform was reported and these mice exhibit diminished recovery from endotoxic shock and hyperresponsiveness of some endotoxin-inducible genes in liver. This is the first in vivo evidence that Stat isoforms have essential in vivo functions (Yoo et al., 2002).

Many different tumor cell lines and fresh isolates derived from a variety of different tumors, including breast, hematopoietic, head and neck, lung, kidney, prostate, and ovarian cancers express activated forms of Stat3 and Stat5 (Bowman et al., 2000; Levy and Gilliland, 2000). These Stats are often activated upon engagement of growth factor receptors and facilitate cellular expansion by transactivating genes encoding proteins that enhance cell survival (such as Bcl-2 and Bcl-X_L). Stat3 and Stat5 are also frequently activated in cells that are transformed with a variety of oncogenes (such as v-src and BCR-abl). Moreover, recent work has shown that enforced expression of a constitutively active, disulfide-linked Stat3 homodimer is sufficient to transform immortalized fibroblasts, as detected by colony formation in vitro and tumor formation in nude mice (Bromberg and Darnell, 2000). Thus, Stat3 and Stat5 function in a manner that promotes growth and/or survival of transformed cells, unlike Stat1, which inhibits these functions. Tumors that express activated Stat3 and/or Stat5 often additionally express activated Stat1. It has been speculated that Stat1 and Stat3 maintain a critical biological balance in normal growth-regulated cells and that perturbation of this balance may result in oncogenesis and/or a transformed cellular phenotype.

Stat4

IL-12 promotes differentiation of naive CD4$^+$ T cells to Th1 cells, which produce IFN-γ and augment cell-mediated immune responses. Th1 cells are critical in host defense against intracellular pathogens and tumors and in the pathogenesis of autoimmune diseases such as rheumatoid arthritis, diabetes, and multiple sclerosis. IL-12 activates Stat4 and the phenotype of Stat4 knockout mice is similar in most respects to mice lacking IL-12 or IL-12R subunits, having impaired Th1 differentiation, IFN-γ production, and cell-mediated immunity (Wurster et al., 2000). Stat4 is also required for proper expression of IL-12R and IL-18R on Th1 cells (Lawless et al., 2000).

As might be predicted, Stat4-deficient mice are resistant to autoimmune diseases characterized by a Th1 response, such as models of arthritis, diabetes, and experimental allergic encephalomyelitis (EAE) (Chitnis et al., 2001). Conversely, IL-12-, IL-12 receptor-, and Stat4-deficient mice have increased susceptibility to infection with intracellular organisms. In acute sepsis though, Stat4 deficiency is associated with improved survival, whereas Stat4$^{-/-}$ mice had *increased* lethality in a noninfectious sepsis model (Lentsch et al., 2001; Matsukawa et al., 2001); apparently, Stat4 may have either pro- or anti-inflammatory effects, depending upon context.

Stat4 is also activated by IL-23, and the exact functions of IL-12 versus IL-23 will need to be sorted out (Oppmann et al., 2000). Additionally, in humans, but not in mice, IFN-α/β induces Stat4 phosphorylation. This is notable because IFN-α/β can promote Th1 differentiation in humans and not in mice. This has been explained by the finding that Stat4 Stat2 is recruited to the human type I IFN receptor via carboxy terminus of Stat2 (Farrar et al., 2000).

Stat4 is also inducible in dendritic cells and macrophages; as in lymphocytes, Stat4 appears to be important for production of IFN-γ in nonlymphoid cells. This has been a controversial issue that now appears to have a mechanistic basis and may be a means by which DC promote Th1 differentiation (Frucht et al., 2001a).

Stat6

Exposure of naive CD4$^+$ cells to IL-4 generates Th2 cells, which are important in host defense against helminthes and allergic responses. Activated by IL-4 and IL-13, Stat6 is critical for Th2 differentiation. Accordingly,

Stat6$^{-/-}$ mice have defective responses to these cytokines (Wurster et al., 2000; Zhu et al., 2001). Stat6 regulates the expression of GATA-3 and c-maf, two transcription factors in involved Th2 function. Th2 differentiation is also associated with remodeling of chromatin in the IL-4 locus and Stat6 is essential for this event (Okamura and Rao, 2001). Additionally, Stat6-deficient B cells are unable to undergo class switching and produce IgE. Via Stat6, IL-4 also antagonizes Th1 responses. In fact, residual Stat4-independent Th1 differentiation becomes apparent in doubly deficient Stat4/Stat6 knockout mice.

As expected, Stat6$^{-/-}$ mice have impaired expulsion of helminthic parasites, reduced pathology in models of asthma, and exaggerated severity of Th1 diseases like EAE (Chitnis et al., 2001). Stat6$^{-/-}$ mice have increased lethality, inflammation, and cytokine production in a noninfectious model of endotoxemia, but reduced lethality and enhanced clearance of bacteria in an infectious model (Lentsch et al., 2001; Matsukawa et al., 2001). Stat6 deficiency is associated with enhanced tumor immunity (Terabe et al., 2000).

Stat5

The products of the closely related (>90% identical) and chromosomally linked Stat5a and Stat5b genes are activated by a wide range of cytokines. It was striking that mice singly deficient in each of these genes had a very specific and limited phenotype. Stat5a knockout mice have loss of Prl-mediated mammary gland development, whereas Stat5b-deficient mice have sexually dimorphic growth retardation. In contrast, many Stat5a/b double knockout mice die within a few weeks of birth, are infertile with defective corpus luteum development, and have defective mammary gland development. Both male and female Stat5a/b-deficient mice are small with reduced size of fat pads and reduced levels of insulin-like growth factor-1 (IGF-1). Stat5a/b double knockout mice also have hypocellular bone marrow, lymphopenia, neutrophilia, and modest anemia and thrombocytopenia (Bunting et al., 2002; Snow et al., 2002). More impressive is the reduced ability of Stat5a/b-deficient hematopoietic progenitors to repopulate marrow, especially in competitive repopulation assays. Myeloid development is grossly normal in Stat5a/b knockout mice, but in vitro cytokine-dependent proliferation, survival, and migration of myeloid cells to sites of inflammation in vivo are impaired (Kieslinger et al., 2000). These mice have reduced numbers of B cell precursors and mature B cells and reduced responsiveness to IL-7, but immunoglobulins are not reduced (Sexl et al., 2000). NK cells are absent but thymic development is intact; however, peripheral T cells from these mice constitutively express activation markers and have impaired in vitro proliferation. Presumably this reflects in vivo activation, a phenotype that is similar to IL-2- and IL-2 receptor-deficient mice and may be the consequence of defective AICD. Stat5a/b$^{-/-}$ mice, like IL-2$^{-/-}$ mice, develop autoimmunity, and this also contributes to their pathology.

Interestingly, Stat5 deficiency abrogates transformation by Tel-Jak but not v-Abl or BCR-abl-mediated transformation (Schwaller et al., 2000; Sexl et al., 2000).

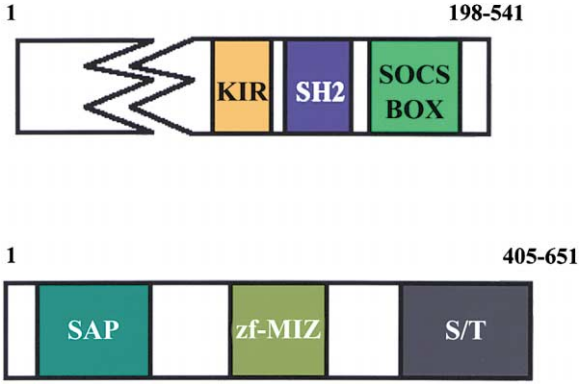

Figure 4. Structure of SOCS and PIAS Family Members

SOCS proteins have a central SH2 domain and a C-terminal SOCS box; SOCS1 and SOCS3 also have a kinase-interacting region (KIR). PIAS proteins contain a SAP (SAF-A/B, Acinus and PIAS) domain, a ring-finger domain and C-terminal serine/threonine rich region.

Negative Regulation Cytokine Signaling

The mechanisms of negative regulation of signaling are the subject of several recent reviews (Krebs and Hilton, 2001; Yasukawa et al., 2000) and will be briefly considered here. Several protein tyrosine phosphatases (PTP) including SHP-1, CD45, and PTP1b have been shown to be negative regulators of cytokine signaling (Irie-Sasaki et al., 2001; Myers et al., 2001). CD45 is a hematopoietic-specific phosphatase, but cytokine receptors, Jaks, and Stats are widely expressed; what phosphatases(s) perform CD45's function in nonhematopoietic tissues? PTP1b binds Jak2 and Tyk2 via the phosphorylated activation loop and inhibits signaling, but Jak1 and Jak3 are apparently not regulated by this phosphatase. After translocation to the nucleus, Stats are dephosphorylated, but the identity of the nuclear Stat phosphatase has not been established.

SOCS/Jab/SSI/CIS

Initially identified as cytokine-inducible inhibitors of cytokines, this family comprises at least eight members with a central SH2 domain and a carboxy terminal "SOCS box" (Figure 4), but only four members, CIS, SOCS1, SOCS2, and SOCS3, have been studied to any extent.

The essential negative regulatory function of SOCS1 is evidenced by the fatal, immune-mediated inflammatory disease present in SOCS1$^{-/-}$ mice. These mice are viable and fertile but die at 3 weeks of age with a disorder characterized by severe lymphopenia, activated T cells, macrophage infiltration, fatty degeneration, and necrosis of the liver. This disorder is present in mice in which the SOCS box is selectively deleted, illustrating its critical function (Zhang et al., 2001). IFN-γ and TNF are produced in high levels in these mice and the acute pathologic changes are attenuated by the lack of IFN-γ, Stat1, Stat6, T cells, and NK T cells (Naka et al., 2001). Over time however, mice lacking both SOCS-1 and IFN-γ develop chronic multiorgan pathology, including polycystic kidneys, pneumonia, skin ulcers, granulomas, and a slightly increased incidence of spontaneous and induced T cell leukemias (Metcalf et al., 2002).

SOCS2-deficient mice exhibit gigantism similar to GH

Table 1. Phenotype of Nullizygous Mice

Gene	Phenotype
Jak1	Viable but perinatal lethality due to neurologic deficits, SCID.
Jak2	Embryonically lethal due to failure in erythropoiesis.
Jak3	Viable and fertile, SCID.
Tyk2	Viable and fertile, defective IL-12 signaling especially in NK cells, increased susceptibility to selected viral infections.
Stat1	Viable and fertile, defective IFNα/β and IFN-γ functions, increased tumorigenicity.
Stat2	Viable and fertile, defective IFN-α/β functions, reduced Stat1 expression in some tissues.
Stat3	Embryonic lethal, conditional knockouts define tissue specific functions (see text).
Stat4	Viable and fertile, defective IL-12-driven Th1 differentiation, increased susceptibility to intracellular pathogens.
Stat5A	Viable and fertile, defective in prolactin functions and mammary gland development.
Stat5B	Viable and fertile, defective in sexually dimorphic growth.
Stat5 A/B	Viable, female infertility, defective mammary gland development, reduced body mass in males and females, defective T cell proliferation.
Stat6	Viable and fertile, defective IL-4-driven Th2 differentiation, increased susceptibility to helminthic infestation.
Stat4/6	Viable and fertile, defective Th-2 differentiation (Th1 skewed).
SOCS1	Viable but perinatal lethality severe IFN-γ-dependent inflammatory disease.
SOCS2	Viable and fertile. Gigantism due to increased growth hormone and IGF-1.
SOCS3	Embryonically lethal due to placental defects and erythrocytosis.
CIS	Viable and fertile — no phenotype reported.

and IGF-1 transgenic mice (Metcalf et al., 2000). In contrast, SOCS3 deficiency is embryonic lethal, but the basis of this lethality is somewhat controversial. One study reported that SOCS3$^{-/-}$ embryos have marked erythrocytosis, consistent with the finding that transgenic expression of SOCS3 resulted in inhibition of erythropoiesis. However, a more recent study indicated that SOCS3 deficiency resulted in placental defects (Roberts et al., 2001).

Despite their overall similarity in structure, SOCS family members apparently have distinct mechanisms of inhibition of signaling. First, SOCS1 and SOCS3 can bind Jaks but the ability to inhibit their catalytic activity is variable. In the case of Jak2, Y1007 in the activation loop binds the SOCS1 SH2 domain (Yasukawa et al., 2000). Additionally though, both SOCS1 and SOCS3 have a region immediately N-terminal to the SH2 domain termed the kinase inhibitory region, which may function as a pseudosubstrate and bind in the Jak catalytic pocket. In contrast, CIS binds to cytokine receptors and blocks STAT recruitment to the receptor; neither CIS nor SOCS2 bind Jaks. Interestingly, SOCS3 binds both the cytokine receptor and the Jak (Nicholson et al., 2000); SOCS3 is recruited to the tyrosine-phosphorylated receptor, facilitating inhibition of the Jak. However, recruitment of SOCS3 may not inhibit all signaling; SOCS3 can also bind RasGAP and consequently enhance Ras signaling (Cacalano et al., 2001). Promotion of protein degradation is a fourth, but not mutually exclusive, mechanism by which SOCS attenuate signaling. The SOCS box binds the elongin B/C complex, which binds an E3-like ubiquitin ligase, cullin-2, and regulates proteosomal degradation. This mechanism of inhibition serves to downregulate transformation by Tel-Jak2 (Frantsve et al., 2001; Kamizono et al., 2001).

PIAS, Stat Methylation, and the SUMO Connection

Protein inhibitor of activated Stat (PIAS)1, identified in a yeast two-hybrid screen (Figure 4), is one of several members of a family that now includes PIAS3 (KchAP), PIASy, and PIASx (ARIP3) (Shuai, 2000). These proteins bind activated Stat dimers and block transcription. However, it is now known that PIAS proteins interact with a variety of different proteins and can serve as E3-like ligases for sumoylation (Jackson, 2001). This begs the obvious questions—are Stats sumoylated, and how might this modification alter Stat function? An additional interesting twist is that inhibition of Arg methylation by methylthioadenosine of Stat1 promotes PIAS binding (Mowen et al., 2001). Transformed cells can lack methylthioadenosine phosphorylase and thereby accumulate methyl thioadenosine; this might impair IFN responses and enhance tumorigenesis.

Conclusions

In this review we have tried to highlight the state of the art of Jaks and Stats, a field of intense study. The vast number of recent publications is indicative of just how quickly this field is moving—and it's not just details. On the contrary, it is absolutely clear that Jaks and Stats are critical regulators of many key processes involving cell fate and differentiation.

A particularly exciting area is the investigation of function of Jaks and Stats in model organisms such as *Dictyostelium* and *Drosophila* (Williams, 2000; Zeidler et al., 2000). Multiple *Dictyostelium* Stats play important roles in the lifecycle of this organism, but no Jak or cytokine receptor has been identified in this organism; in this case, it appears that entirely different types of receptors are involved (Fukuzawa et al., 2001). A "complete Jak/Stat" pathway is present in *Drosophila* (Zeidler et al., 2000) and recently a cytokine receptor has also been identified. Termed *domeless or mom*, it is similar to LIFR and CNTFR (Brown et al., 2001) and its ligand is a secreted molecule designated *unpaired*. This pathway also has been shown to be critically involved in processes such as embryonic segmentation, sex determination, larval hematopoiesis, eye, wing and leg development, stem cell maintenance, and ovarian cell migration (Jinks et al., 2000; Kiger et al., 2001; Sefton et al., 2000; Silver and Montell, 2001; Tulina and Matunis, 2001; Zeidler et al., 2000). This is surely an exciting area of investigation.

While Jak- and Stat-deficient humans and mice dra-

matically illustrate the essential in vivo functions of these molecules, an important question in the field is to what extent, if any, Jaks and Stats are important for signaling for non-Type I/II cytokines. Virtually all classes of ligands and receptors have been reported to activate Jaks and Stats, but are they essential components for signaling? This is still an open question; the data from *Dictyostelium* would argue that Stats at least, have important functions for non-cytokine receptors, but is this also the case for Jaks? Another important issue is that many cytokines activate multiple Stats. Given the very different functions of the Stats, it will be of interest to determine the significance of these findings. Additionally, the finding of many IFN-inducible genes in Stat1-deficient cells illustrates that important Stat-independent signals exist. Do other Stats provide compensatory signals, or is the control of these genes entirely unrelated to Stats? Clearly these findings provide impetus to try to sort out the mechanisms that control these genes.

A major developing area of unquestioned physiologic relevance is that viruses possess a number of mechanisms that circumvent IFN action and interdict Jak/Stat signaling, with different viruses using distinct mechanisms. Mechanisms identified thus far include destabilization of Jak and Stat proteins and perhaps other transcription factors (Levy and Garcia-Sastre, 2001; Parisien et al., 2001). Conversely, IFNs circumvent virus-imposed cellular inhibitions; for instance, IFNs upregulate nucleoporins to release vesicular stomatitis virus-mediated mRNA nuclear export block (Enninga et al., 2002). Furthermore, poliovirus promotes degradation of TATA binding protein (TBP); however, IFN-dependent transcription functions independently of TBP and thus can evade the viral block (Paulson et al., 2002). This work is just beginning to appear in literature, but the interplay between viruses and elements in IFN signaling will, no doubt, continue to receive a lot of attention in the future. This is also an area that might be capitalized upon for the development of novel therapies.

Finally, immense progress continues to be made in identifying mechanisms involved in chromatin modification and how these alterations promote or repress transcription (Jenuwein and Allis, 2001). It is clear that cytokine signaling leads to chromatin remodeling, but what are the precise steps that govern this process? This is sure to be an exciting area of investigation.

These concluding statements highlight just a few emerging areas of provocative work. It is clear that this continues to be an exciting field; there are plenty of excellent tools and no shortage of fascinating areas for study.

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