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JAKS AND STATS: Biological Implications*

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ABSTRACT

Cytokines and interferons are molecules that play central roles in the regulation of a wide array of cellular functions in the lympho-hematopoietic system. These factors stimulate proliferation, differentiation, and survival signals, as well as specialized functions in host resistance to pathogens. Although cytokines are known to activate multiple signaling pathways that together mediate these important functions, one of these pathways, the Jak-STAT pathway, is the focus of this chapter. This pathway is triggered by both cytokines and interferons, and it very rapidly allows the transduction of an extracellular signal into the nucleus. The pathway uses a novel mechanism in which cytosolic latent transcription factors, known as signal transducers and activators of transcription (STATs), are tyrosine phosphorylated by Janus family tyrosine kinases (Jaks), allowing STAT protein dimerization and nuclear translocation. STATs then can modulate the expression of target genes. The basic biology of this system, including the range of known Jaks and STATs, is discussed, as are the defects in animals and humans lacking some of these signaling molecules.

OVERVIEW

Cytokines and interferons are intercellular messengers that induce many important biological responses in target cells (1). Over the past five years, an

extraordinarily exciting new signaling pathway has been elucidated. This pathway involves families of proteins, denoted as Jaks (Janus family tyrosine kinases) and STATs (signal transducers and activators of transcription) (2–7). This pathway has proved to be integral to both type I (IFNα/β) and type II (IFNγ) interferons (collectively also known as type II cytokines) and to all cytokines whose receptors are members of the cytokine receptor superfamily, also known as type I cytokine receptors. These type I cytokines include the short-chain cytokines, IL-2, IL-3, IL-4, IL-5, GM-CSF, IL-7, IL-9, IL-13, and IL-15 and the long-chain cytokines, IL-6, IL-11, OSM, CNTF, CT-1, growth hormone, prolactin, erythropoietin, and thrombopoietin. These cytokines form a family based on sharing a similar four-α-helical bundle structure (reviewed in 1, 8). The Jak-STAT pathway represents an extremely rapid membrane-to-nucleus signaling system and clarifies at least part of the basis for the specificity of signals that are induced by different cytokines.

JANUS KINASES AND CYTOKINE SIGNAL TRANSDUCTION

Discovery of the Jaks

Four mammalian Jaks have been identified: Jak1, Jak2, Jak3, and Tyk2. This new class of kinases was discovered by two approaches. Tyk2 was first identified by low-stringency hybridization screening of a T cell cDNA library with the c-fms catalytic domain (9), whereas the remainder of the Jaks (Jak1, Jak2, and Jak3) were cloned with a PCR-based strategy using primers corresponding to conserved motifs within the catalytic domain of tyrosine kinases (10–16). Carp and zebrafish Jak1 have also been cloned (17, 18). A Drosophila Jak was identified in the analysis of mutant flies with defects in the expression patterns of the pair-rule genes and segment-polarity genes, giving the gene its name, *hopscotch* (19).

Characteristics of Jaks

Jaks are relatively large kinases of approximately 1150 amino acids with apparent molecular weights of about 120–130 kDa. Their mRNA transcripts range from 4.4 to 5.4 kb in length. Jak2 has two transcripts, and multiple spliced forms of Jak3 have been identified, including a variant that lacks a segment of the catalytic domain (12, 16, 20). The functional significance of these variant transcripts is not understood, but it is intriguing to speculate that a naturally occurring dominant negative form of Jak3 may have regulatory function.

In contrast to the relatively ubiquitous expression of Jak1, Jak2, and Tyk2, Jak3 has more restricted and regulated tissue expression. It is expressed constitutively at high levels in NK cells and thymocytes and is inducible in T cells,

B cells, and myeloid cells (14, 16, 21–23). In addition to its expression in hematopoietic cells, Jak3 is also expressed in vascular smooth muscle cells and endothelium (24). The mechanisms by which Jak3 expression is regulated have not been well studied, but it is notable that the putative Jak3 promoter found in the mouse gene contains consensus binding sites for a variety of transcription factors, which may explain the inducibility of the Jak3 gene (25).

Genomic Organization and Chromosomal Localization

The intron/exon organization of the murine Jaks, human Jak3, and carp Jak1 has been determined (17, 25–28). The genomic organization is conserved among murine genes for different Jaks, but the intron/exon organization of the Jaks does not conform to the defined Jak homology domains of the polypeptides. As is later discussed, a unique feature of the Jaks is the presence of a pseudokinase domain in conjunction with the bona fide catalytic domain. The organization of the kinase and pseudokinase domains is not conserved, suggesting distinct evolutionary origins.

In humans, the Jak1 gene resides on chromosome 1p31.3 and Jak2 at 9p24 (reviewed in 3). Interestingly, Jak3 and Tyk2 are nearby on chromosome 19; Jak3 maps to 19p13.1 and Tyk2 maps to 19p13.2 (26, 27, 29). The murine genes reside on chromosomes 4 (Jak1), 19 (Jak2), and 8 (Jak3) (30).

STRUCTURE OF JANUS KINASES

The hallmark of the Jak family of PTKs is the existence of tandem kinase and pseudokinase domains, a feature that also gives the Janus kinases their name (Figure 1). Like the Roman god of gates and doorways, the Janus kinases are "two-faced." In addition, several other segments of homology were recognized among the Jaks, and hence Jak homology (JH) domains were defined using nomenclature analogous to that of the Src homology (SH) domains of Src family PTKs (10, 11). Seven JH regions (JH1-JH7) are described, but it is important to emphasize that, with the exception of the JH1 or catalytic domain, the function of these regions remains poorly understood. Moreover, which JH regions are, in fact, independent structural domains remains unclear.

The JH1 or Catalytic Domain

The JH1 domain has all of the features of a typical tyrosine kinase domain and, as with other tyrosine kinases, mutation of the conserved Lys residue in subdomain II that binds ATP abrogates kinase activity (16, 31–35).

Tyrosine residues in the activation loop of PTKs typically play an important role in regulating catalytic activity (36). Structural studies support a model in which, prior to phosphorylation, the activation loop impedes substrate access

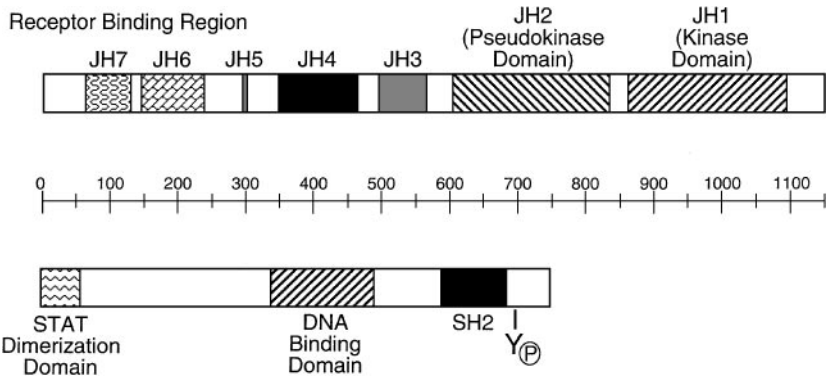

Figure 1 Structure of Jaks and STATs. Jaks are structurally unique in having tandem kinase and kinase-like domains. The C-terminal kinase domain is the catalytic domain, and the precise function of the kinase-like domain has yet to be determined. Regions of homology shared by Jaks have been termed Jak homology (JH) domains. Like Src homology (SH) domains, they are named in a C-terminal–to–N-terminal direction. The JH1 domain is the kinase domain and the kinase-like domain is the JH2 domain. The remainder of the homology domains are indicated, though their functions are not well understood. The N-terminus of the Jaks, however, appears to be important for association with cytokine receptor subunits. STATs have a conserved tyrosine whose phosphorylation allows STAT dimerization, an SH2 domain that mediates the dimerization, and an N-terminal region known to play a role in the dimerization of STAT dimers. Interacting proteins bind at both the N-terminal and C-terminal regions of STATs. In some STATs, a serine phosphorylation site has been identified C-terminal to the conserved tyrosine.

to the active site of the enzyme, whereas phosphorylation of tyrosine residue(s) within the loop facilitates substrate accessibility. Consistent with this model, mutation of such residues (e.g. Tyr 1162 of the insulin receptor kinase) reduces kinase activity. Multiple autophosphorylated sites have been identified in Jak kinases, including sites within the activation loop (33–35). Mutation of Tyr residues in the activation loop of the Jaks, though, has variable effects on catalytic activity. For Jak2, mutation of Tyr 1007 to Phe abrogated kinase activity and the ability to transmit cytokine-mediated signals (34). However, mutation of the corresponding Tyr residues in Tyk2 (Tyr 1054 and Tyr 1055) blocked ligand-dependent tyrosine phosphorylation of Tyk2 but did not abolish its kinase activity (33). Indeed, mutations either in the activation loop or in the ATP binding site reduced but did not abrogate signaling, while mutations at both sites abolished signaling. Like Tyk2, mutation of Y980 in Jak3 inhibited but did not abolish kinase activity. In contrast, mutation of Y981 augmented kinase activity (35). Mutation of the corresponding Tyr residue in Jak2, however, did not enhance catalytic activity. Thus, surprisingly, there may be substantial differences in the regulation of catalytic activity of the different Jaks. The

consequence of the mutation of individual Tyr residues in the activation loop of Tyk2 has not yet been reported.

The Pseudokinase or JH2 Domain

Among metazoan PTKs, only the Jaks have a pseudokinase domain, and the function of this region has not yet been determined. The pseudokinase domain has all of the subdomains that correspond to those in bona fide tyrosine kinase catalytic domains, but they are altered from the typical motifs. For example, JH2 domain lacks the third Gly of the canonical GXGXXG motif (subdomain I). Also lacking is a nearly invariant aspartic acid residue that is present in both tyrosine and serine kinases in the catalytic loop (subdomain VIb) that serves as the proton acceptor in the phosphotransfer reaction. The JH2 domain also lacks a conserved Phe residue (in the motif Asp-Phe-Glu in subdomain VII) that is important for binding the adenine ring of ATP. The lack of these key residues suggested that the pseudokinase domain would not have catalytic activity, and this has been confirmed (10, 16, 37, 38). However, several lines of evidence indicate a role for JH2 domains in regulating Jak catalytic function. In the context of a growth hormone receptor/Jak chimera, deletion of the JH2 domain led to more robust signaling, leading the authors to speculate that the JH2 domain serves to inhibit Jak kinase activity (39). Consistent with this possibility, a gain-of-function mutation has been found in the JH2 domain of the *Drosophila* Hopscotch Jak kinase (40), and a similar mutation in Jak2 is also activating. One can envision a structural model in which the JH2 domain impedes access to the kinase domain; activation would entail relief of this inhibition. However, other data suggest other possible roles for the JH2 domain. For Tyk2, removal of the pseudokinase domain abrogated kinase activity and IFNα/β-induced signaling. In addition, several patients with nonfunctional Jak3 (see below) have mutations in this domain (41). Thus, at present the precise functions of the JH2 domain remain unclear. Clearly, structural information pertaining to the interactions of the JH1 and JH2 domains will be of great interest. Another potential function of JH2 is a docking site for other signaling molecules. Indeed, the JH2 domain associates with STATs (42).

Dictyostelium PTKs (DPYK3 and DPYK4) have been cloned that have tandem kinase domains (43), but overall they are not highly homologous Jaks, even though a Dictyostelium STAT has been identified (44).

The Jak N-Terminus: Role In Cytokine Receptor Binding

Though a complete understanding of the function of the relatively divergent N-termini of the Jaks is presently lacking, one function that has been defined is the ability of this segment to bind to cytokine receptors (38, 39, 45–47). Whereas deletion of kinase and kinase-like domains, in general, has little effect

on binding, deletion of the N-terminal region eliminates binding. For Jak3, the JH7 and JH6 domains are necessary and sufficient for binding to its cognate cytokine receptor subunit, γ_c. Studies using chimeric Jak constructs (46, 47) support the importance of the Jak N-terminus in binding to cytokine receptors, though the understanding of the basis of Jak/cytokine receptor interactions is far from complete.

The region of cytokine receptors that bind the Jaks has been better characterized. The relatively conserved membrane proximal domain of cytokine receptors is the region that interacts with Jaks and is essential for signal transduction (reviewed in 3). Although this segment is better characterized than the corresponding region of the Jaks, we still do not understand the rules by which a given cytokine receptor recruits a given Jak.

Interestingly, the Jaks have an SH2-like segment in the JH4 domain that has a conserved Arg residue corresponding to the phosphotyrosine binding pocket of other SH2 domains (11). Whether this domain is capable of binding phosphotyrosine has not been demonstrated, however. Moreover, this residue has been mutated with no clear effect on signaling (46).

JANUS KINASES AND CYTOKINE SIGNAL TRANSDUCTION

Shortly after the discovery of the Jaks, their essential function in cytokine signaling was established in a series of experiments using mutagenized cell lines that were resistant to the effects of IFNs. Signaling was reconstituted by transfection of the cells with different Jaks found to be lacking in the cells. Specifically, IFNα/β signaling required Jak1 and Tyk2, whereas IFNγ required Jak1 and Jak2 (2, 48–51). Subsequently, growth hormone and erythropoietin were shown to activate Jak2 (52, 53), whereas IL-6 activated Jak1, Jak2, and Tyk2 (54). In contrast, Jak3 was activated only by cytokines whose receptors contain γ_c (IL-2, IL-4, IL-7, IL-9, and IL-15) and in cells lacking Jak3, IL-2 and IL-4 signaling is compromised (15, 55–60). In subsequent studies, all of the type I cytokines examined activated Jaks (1, 3, 61). These studies are summarized in Table 1.

That Jaks and cytokine receptors associate was shown first by the interaction of Jak2 with the erythropoietin and growth hormone receptors (52, 53). Jak2 also associates with β_c, a shared subunit of the IL-3, IL-5, GM-CSF receptors (62). The gp130 subunit of the IL-6 family of receptors is bound somewhat indiscriminately by Jak1, Jak2, and Tyk2 (54). In contrast, Jak3 specifically associates with γ_c (56, 63, 64), whereas the unique receptor subunits of this subfamily of cytokines (e.g. IL-2Rβ) associate with Jak1 (56). Similarly, for the IFNα/β receptor, the α subunit, also termed IFNAR-1, is associated with

Table 1 Jaks and STATs that are activated by cytokines

Type I Cytokines	Jaks	STATs
Cytokines whose receptors share γ_c		
IL-2, IL-7, IL-9, IL-15	Jak1, Jak3	Stat5a, Stat5b, Stat3
IL-4	Jak1, Jak3	Stat6
IL-13*	Jak1, Jak2, Tyk2	Stat6
Cytokines whose receptors share β_c		
IL-3, IL-5, GM-CSF	Jak2	Stat5a, Stat5b
Cytokines whose receptors share gp130		
IL-6, IL-11, OSM, CNTF, LIF, CT-1	Jak1, Jak2, Tyk2	Stat3
IL-12+	Jak2, Tyk2	Stat4
Leptin+		Stat3
Cytokines with homodimeric receptors		
Growth hormone	Jak2	Stat5a, Stat5b, Stat3
Prolactin	Jak2	Stat5a, Stat5b
Erythropoietin	Jak2	Stat5a, Stat5b
Thrombopoietin	Jak2	Stat5a, Stat5b
Type II Cytokines		
Interferons		
IFNα, IFNβ	Jak1, Tyk2	Stat1, Stat2
IFNγ	Jak1, Jak2	Stat1
IL-10‡	Jak1, Tyk2	Stat3

*IL-13 does not share γ_c but uses IL-4Rα.
+IL-12 and leptin do not share gp130, but their receptors are related to gp130.
‡IL-10 is not an interferon, but its receptor is a type II cytokine receptor.

Tyk2, and the β subunit (IFNAR-2) is associated with Jak1 (65–67). By comparision, the IFNγRα subunit (IFNGR-1) associates with Jak1 and IFNγRβ (IFNGR-2) associates with Jak2 (reviewed in 5). For the IL-12R, the β1 subunit associates with Tyk2, while the β2 subunit associates with Jak2 (68). Importantly, although specific Jaks are recruited to different cytokine receptors, this is not an important mechanism by which specificity in cytokine signaling is imparted. First, Jak usage is too degenerate to provide such specificity (e.g. many different cytokines activate Jak1 and Jak2; see Table 1). Second, experiments have shown that artificially recruiting a different Jak to a receptor does not alter signaling (69). Finally, although Jaks are constitutively associated with receptors, a ligand-inducible augmentation is seen in several receptor systems; the mechanism underlying this augmentation has not been identified.

Jaks therefore satisfy two key criteria that would be demanded of a proximal signal transducing unit used by cytokine receptors: They physically associate with receptor subunits, and they are essential components for signaling.

Models of Jak Activation

Cytokine receptors form homo- or heterodimers following ligand binding, and the dimerization of cytokine receptor subunits is sufficient to initiate signaling (70–73). Because the Jaks are associated with receptors, they likely become activated by being brought into proximity by receptor hetero- or homodimerization. For cytokine receptors that homodimerize (e.g., growth hormone and erythropoietin receptors), this effectively results in homodimerization of Jak2. Similarly, for receptors that form heterodimers (i.e., most of the interleukin and the interferon receptors), presumably heterodimerization of different Jaks occurs. In these cases, the data have been interpreted to indicate that Jaks are interdependent in their activation. Support for this model comes from studies in which the absence of a given Jak results in the failure of the other Jak to become activated following ligand stimulation. For example, in cells lacking Jak1, no phosphorylation of Tyk2 or Jak2 was observed upon stimulation with IFNα or IFNγ, respectively (50). Conversely, no phosphorylation of Jak1 was seen in cells lacking Tyk2 or Jak2. Similar results have been obtained with IL-2 signaling in Jak3-deficient cells; in the absence of Jak3, no phosphorylation of Jak1 occurred in response to IL-2 (59).

Consistent with this model, reconstitution of Jak1-deficient cells with catalytically inactive Jak1 did not support signaling or IFNα-induced gene expression (32). Accordingly, reconstitution of Jak2-deficient cells with a catalytically inactive form of Jak2 did not permit IFNγ-induced phosphorylation of Jak1 or Jak2.

Surprisingly, however, reconstitution of Tyk2-deficient cells with kinase-dead Tyk2 did permit IFNα/β-induced phosphorylation of Tyk2 and suboptimal signaling (33). Similarly, in Jak1-deficient cells reconstituted with kinase-dead Jak1, IFNγ could still induce suboptimal Jak2 phosphorylation, low-level receptor phosphorylation, STAT activation, and ligand-induced gene expression. The model proposed for IFNγ signaling is that Jak2 phosphorylates itself and Jak1. Jak1 is principally responsible for receptor phosphorylation, Stat1 is recruited to the receptor, and Jak2 then phosphorylates Stat1 (32).

IL-6 signaling is unusual in that three different Jaks (Jak1, Jak2, and Tyk2) are activated. Interestingly, however, the absence of any one Jak does not interfere with the activation of the others, but the absence of Jak1 inhibited IL-6-induced expression of the IRF-1 gene and phosphorylation of gp130, Stat1, and Stat3 (31).

Thus, although the Jaks are to an extent interdependent, they are not functionally symmetrical. Given the aforementioned differences emerging in the catalytic regulation of the Jaks, the non-equivalence of the Jaks is not surprising. The data also argue for a structural role of Jaks beyond an enzymatic role.

Attenuation of Jak Signaling

Reversible Jak activation is quite evident following cytokine stimulation; however, the mechanisms by which the termination of Jak signaling is accomplished are poorly understood. The SH2-containing tyrosine phosphatase, Shp-1, associates with some cytokine receptors and regulates Jak phosphorylation (74, 75). Whether other cytokine receptors and Jaks are regulated similarly has not been documented. Jaks are also associated with Shp-2 (76), but in general Shp-2 positively regulates signaling (see below) and does not attenuate it (77).

Interestingly, an inhibitor of Jak catalytic activity has recently been identified (78–80). This protein, variably termed SOCS-1 (suppressor of cytokine signal-1), JAB (Jak-binding protein), and SSI-1 (STAT-induced STAT inhibitor-1), is related to originally identified family member CIS (cytokine inducible SH2-containing protein) (81); a total of seven members of this family have now been identified, although the biological actions of each are still being clarified (182). Unquestionably, the mechanisms by which Jak signaling is turned off form an area that deserves more attention.

JAKS AND DEVELOPMENT

Jak3 and Severe Combined Immunodeficiency

While the analysis of mutant cell lines established the essential function of Jaks in cytokine signaling, the significance to the organism of a given Jak has been less well characterized. Thus far, we only have information on organisms deficient in one mammalian Jak, Jak3 (Table 2). Mutation of γ_c, which specifically associates with Jak3, is the molecular basis of X-linked severe combined immunodeficiency (X-SCID). Its intimate association with Jak3 suggested that mutations of the latter might also cause SCID (56, 82, 83). Consequently, the initial patients with autosomal recessive SCID due to Jak3 mutations were identified (58, 84). Subsequently, other patients, including patients with missense mutations, have also been identified (41). Jak3 knockout mice have been generated, and they too are immunodeficient (85–87). Thus, the essential role of Jak3 in lymphoid development is seen in both humans and mice with deficiency of Jak3, although the phenotypes of the immunodeficiency are somewhat different. Whereas Jak3- and γ_c-deficient humans have profoundly decreased numbers of T cells with normal or increased numbers of B cells, Jak3- and γ_c-deficient mice accumulate somewhat larger numbers of peripheral T cells but essentially lack conventional B cells (88, 89). The block in lymphocyte development in Jak3- and γ_c-deficiency presumably relates, at least in part, to the absence of IL-7

Table 2 Phenotypes of mice deficient in various Jaks and STATs

Jaks	
Jak1:	Not yet reported
Jak2:	Not yet reported
Jak3:	Severe Combined Immunodeficiency similar to X-linked SCID
Tyk2:	Not yet reported
STATS	
Stat1:	Defective signaling in response to type I and type II IFNs
Stat2:	Not yet reported
Stat3:	Fetal lethal. Implantation occurs, but fetal growth is blunted
Stat4:	Defective Th1 development, consistent with the role of IL-12 in activating Stat4 and promoting Th1 development
Stat5a:	Defective lobuloalveolar development in the breast
Stat5b:	Required for sexual dimorphism of body growth rates, similar to Laron-type dwarfism, a human disease due to growth hormone resistance. Defective GM-CSF signaling in bone marrow–derived macrophages. Defective IL-2-induced IL-2Rα expression in splenic T cells
Stat6:	Defective Th2 development, consistent with the role of IL-4 for Stat6 and Th2 development

signaling. The difference in B cell development between mice and humans is presumably indicative of an essential role for IL-7 as a pre-B cell growth factor in mice but not humans.

The T cells produced in γ_c- and Jak3-deficient mice are abnormal in that they express activation markers, i.e. high levels of CD44 and low levels of CD62L (90–92). lmpaired negative thymic selection has been reported in Jak3-deficient mice (92), but a complete explanation of the abnormal T cell phenotype in Jak3- and γ_c-deficient mice has not yet been provided. It will be important to more rigorously compare and contrast the effects of γ_c deficiency and Jak3 deficiency in terms of the T cell defect. If γ_c-deficient mice do not have the same defects as Jak3-deficient mice, that would argue that Jak3 has other functions unrelated to γ_c-mediated signaling. For instance, it has been reported that Jak3 associates with CD40, but a specific role for Jak3 in this pathway has yet to be demonstrated (93). Interestingly, the clinical presentation of one patient with a Jak3 missense mutation differed from others in that T cell production occurred over time. The T cells were not normal, however, in that they expressed activation markers, as do Jak3-deficient mice (41).

It is pertinent to note that although Jak3 is inducible in activated monocytes, Jak3-deficient individuals do not have abnormalities attributable to defects in myeloid function; their defects are limited to lymphoid cells (28). The fact that the defects seen in Jak3-deficient individuals are restricted to the immune system has suggested that targeting Jak3 (58, 84) or the interaction between Jak3 and

γ_c might be a useful strategy to generate a novel class of immunosuppressant drugs (56).

Jak1 and Teleost Embryonic Development

Although, Jak3 is the only Jak for which knockout mice have been reported, zebrafish Jak1 was recently cloned and shown to play an important role in early vertebrate development (18). It is maternally encoded, stored in unfertilized eggs, and essential for proper embryogenesis. Injection of RNA-encoding a dominant-negative Jak1 allele interfered with anterior structure formation. These data suggest that Jaks are likely to have important developmental functions in mammal.

Hopscotch and Drosophila Development

Another system that graphically demonstrates the importance of the Jaks in development is the analysis of the function of the *Drosophila* Jak, Hopscotch (Hop) (94). Mutation of the *hop* gene results in lethal segmentation defects through both maternal and zygotic effects with stripe-specific defects in the expression patterns of pair-rule genes (*even-skipped, runt,* and *fushi tarazu*) and segment-polarity genes (*engrailed* and *wingless*) (19). It will be important in the future to define *Drosophila* cytokines, cytokine receptors, and other Jaks if they exist; dissection of these pathways in *Drosophila* should provide considerable insight into the vertebrate pathways.

JAKS AND TRANSFORMATION

Studies in *Drosophila* provide clear evidence of the essential function of the Jaks in normal growth and development. Interestingly, this system also provides evidence that dysregulation of Jaks can lead to cancer. These mutations, known as *Tum-l* or tumorous lethal, result in leukemia in the flies (95–97). This is characterized by formation of melanotic tumors and hypertrophy of larval lymph glands, the hematopoietic organs. Interestingly, overexpression of wild-type *hop* also leads to the formation of tumors. There are a number of circumstances in which constitutive activation of Jaks has been seen in association with malignant transformation in mammalian cells. This was first demonstrated in HTLV-I–transformed T cells (98). Subsequently, constitutive Jak activation has been found in other settings including: Sezary's syndrome (99), *v-abl*-transformed cells (100) and acute lymphoblastic leukemia (ALL) (101). Moreover, a translocation in a patient with T-cell ALL resulted in a Jak2 fusion protein with constitutive kinase activity, and this was transforming in vitro (183). It will be important to understand the mechanism of activation of Jaks in these circumstances and to determine if Jak activation is an essential part of malignant transformation in these cells.

JAKS AND THEIR SUBSTRATES

Presently, our understanding of the relevant Jak substrates is quite limited. The paradigm emerging is that one class of substrate, aside from the kinases themselves, is cytokine receptors. As is discussed shortly, tyrosine phosphorylation of receptors forms docking sites for proteins with phosphotyrosine binding domains, which in turn are also Jak substrates. The STAT family of transcription factors is one example, and the adaptor molecule Shc is another. Other proteins that reportedly interact with Jaks include Grb2, SHP-2, Vav, and STAM (76, 102–104).

In summary, in the short time since their discovery, Janus kinases have been shown to have essential functions in cytokine signaling and development. In humans, the absence of one Jak, Jak3, results in profound immunologic disease. In Teleosts and Drosophila, Jaks clearly have essential roles in early development. A role for Jaks in oncogenesis is also apparent. Jaks are, however, only part of the story. The discovery of the STATs provides important insights as to how extracellular signals effect gene expression.

STATS

The seven known mammalian STAT proteins are denoted Stat1, Stat2, Stat3, Stat4, Stat5a, Stat5b, and Stat6 (105–117). Most of the STATs are approximately 750 to 800 amino acids in length, whereas Stat2 and Stat6 are approximately 850 amino acids in length. The STATs exist as clusters on chromosomes, with Stat1 and Stat4 on mouse chromosome 1, Stat2 and Stat6 on mouse chromosome 10, and Stat3, Stat5a, and Stat5b on mouse chromosome 11 (118). Stat5a and Stat5b are closely positioned on human chromosome 17 (116). The clustering similarities on human and murine chromosomes are consistent with a common ancestral gene that was initially duplicated, followed by subsequent duplication events. The facts that Stat2 and Stat6 are both of the longer STAT form and that they colocalize are consistent with this hypothesis.

Mechanism of Activation of STATs

Following the binding of cytokines or interferons to their receptors, STAT proteins can be activated. The basic scheme of STAT protein activation is summarized in Figure 2 and has been reviewed previously (2–7). The binding of a cytokine to its cognate receptor rapidly induces the tyrosine phosphorylation of the receptor by Jak kinases. Such phosphorylated tyrosines provide docking sites for STATs. The STATs themselves are phosphorylated, released from the receptor, and then can dimerize. The dimeric form can then translocate into the nucleus, where it modulates expression of target genes. A remarkable feature of this system is that newly induced STAT DNA binding activity can be detected in the nucleus within minutes of cytokine binding. Thus, in accord with the

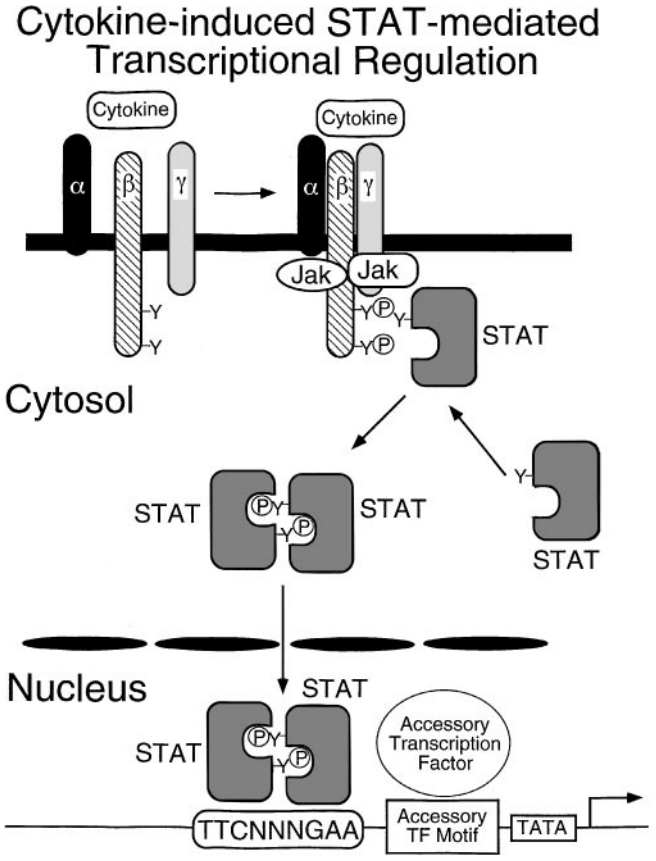

Figure 2 A model for cytokine signal transduction. In this model of the IL-2 and IL-15 receptors, there are three receptor subunits, two of which interact with Jak kinases. The addition of the cytokines activates the Jaks, The Jaks in turn phosphorylate tyrosine-based docking sites on the receptor. STATs then bind via their SH2 domains. The STATs are then phosphorylated, form homo- or heterodimers, and then translocate to the nucleus, where they bind target sequences. Shown is a γ-IFN activated sequence motif (see text). In at least some cases, GAS motifs can be multimers, and STAT dimers can associate with adjacent STAT dimers to allow higher affinity binding. Transcriptional activation of genes may also require accessory transcription factors.

urgency of an emergency "STAT" order in clinical medicine, STATs have an appropriate acronym that accurately reflects the rapidity of their activation and abilities to exert biological actions.

As indicated above, STATs have six essential functional requirements. These are the abilities (*a*) to bind phosphorylated tyrosines, (*b*) to themselves become tyrosine phosphorylated, (*c*) to dimerize, (*d*) to translocate into the nucleus, (*e*) to bind DNA, and (*f*) to modulate gene expression.

Docking of STATs on Receptor Molecules

The bases for some of these functions are well understood. As shown in Figure 1, STAT proteins have a conserved SH2 domain (typically between residues 600 and 700) that mediates the first function. One mechanism for specificity derives from the fact that the SH2 domains of different STATs differ sufficiently so that they recognize different phosphorylated motifs. For example, Stat6 binds to phosphotyrosine docking sites on the IL-4 receptor α chain (117), whereas Stat5a and Stat5b bind to related but distinct docking sites on the IL-2 receptor β chain (IL-2Rβ) and IL-7 receptor α chain (IL-7Rα) (119). The STATs that are activated by various cytokines are summarized in Table 1. In some situations, docking may not be on a receptor chain. Indeed, in the case of IFNα/β stimulation, it is believed that while Stat2 docks on the receptor, Stat1 docks on Stat2 following the tyrosine phosphorylation of Stat2 (120). In addition to docking on receptor chains and other STATs, Jak kinases also provide docking sites. Indeed, STAT5 proteins can coprecipitate with JAK3, suggesting that a direct interaction can occur (42, 98).

Phosphorylation and Dimerization of STATs

Each of the STATs has a conserved tyrosine residue approximately 700 residues from the N-terminus, slightly distal to the end of SH2 domain, that is a substrate for phosphorylation (6). Following its tyrosine phosphorylation, the STAT can form homodimers or heterodimers based on the interaction between the SH2 domain on one STAT molecule and the phosphorylated tyrosine on another. Indeed, as each STAT has both an SH2 domain and a phosphorylated tyrosine, these dimers are stabilized by bivalent interactions. The bivalent nature of these interactions helps to explain why dimerization of STATs is favored over the monovalent interaction of the STAT SH2 with a phosphorylated receptor. Some cytokine receptor chains, such as the IFNGR-1 (also denoted the IFNγRα chain) (121) and IL-7Rα (119), have single STAT docking sites. Others, such as IL-2Rβ (119, 122), IL-4Rα (117), and gp130 (a common receptor chain shared by the receptors for IL-6, IL-11, OSM, CNTF, LIF, and CT-1 (123), have more than one. The presence of more than one docking site allows for the possibility that two STAT molecules might simultaneously be activated (i.e., phosphorylated) in close proximity to each other, thereby potentially facilitating their dimerization. Whether homodimerization or heterodimerization occurs is based on the specificity of the SH2 domain and the microenvironment of the phosphorylated tyrosine. Thus, some heterodimerization events such as Stat1 with either Stat2 or Stat3 (6) and Stat5a with Stat5b (113), can occur, whereas others do not occur. Furthermore, Stat2 is so far known to exist as a heterodimer with Stat1, but not as a homodimer.

As indicated above, for IFN and type I cytokine receptors, the kinases that phosphorylate the STATs appear to be Jak kinases. However, certain growth factors, such as epidermal growth factor and platelet-derived growth factor, bind to receptors with intrinsic tyrosine kinase domains and can activate STATs. Thus, other kinases besides Jaks may also be able to mediate the activation of STATs.

Nuclear Translocation and DNA Binding of STATs

Although the dimerization presumably unmasks a nuclear localization signal (NLS), the residues comprising the STAT NLS are not well defined. It is therefore also unclear whether the STATs enter the nucleus alone or in the context of chaperonin molecules, and whether their nuclear localization requires the release of a cytosolic anchoring protein (e.g., a functional analog of IκB whose association with NF-κB retains NF-κB in the cytosol, discussed below). Once in the nucleus, in general, the STAT homodimers or heterodimers can directly bind to DNA. However, this is not the case for the signaling complex activated by type I interferons (IFNα and IFNβ). Type I interferons activate a Stat1-Stat2 heterodimer that requires a DNA binding protein, p48, to bind DNA (6). p48 is a member of the IRF family of proteins. Perhaps not surprisingly, then, this Stat1-Stat2-p48 complex recognizes a relatively nonpalindromic, interferon-stimulated response element (ISRE) motif, AGTTTNCNTTTCC (6), whereas the other STAT complexes tend to bind semipalindromic motifs, TTCNNNGAA or TTCNNNNGAA, although some variation is allowed even in the TTC/GAA flanking sequences. These semipalindromic motifs are known as GAS motifs, for IFNγ-activated sequences, based on their original identification for the sequences recognized by IFNγ-induced Stat1 homodimers; however, it is now clear that such sequences can be recognized by multiple other STATs as well. The number of nucleotides in the central core of the motifs depends on the particular STAT combination. This is determined by a 180-amino-acid-long DNA binding domain centrally located within STATs (124, 125). The DNA binding domains were delineated by using chimeric constructs that created fusions between different STAT proteins with different specificities so that it could be determined when one specificity was lost and another gained.

Transcriptional Activation by STATs

The sixth function of STATs listed above was the ability to modulate gene expression. In principle, depending on the context, individual transcription factors can either activate or repress the transcription of target genes; however, at least so far, STATs have been identified only in the setting of gene activation and augmented transcription. For some STATs, such as Stat1 (6), Stat2 (126), and

Stat5 (127), C-terminal transcriptional activation domains (distal to the conserved phosphorylated tyrosine) have been delineated. For Stat1 and Stat3, it is known that the C-terminal regions contains a serine residue (at residue 727 for both Stat1 and Stat3) whose phosphorylation is important for potent activity (128, 129). Stat4 has an analogous serine residue. IL-12-induced serine phosphorylation of Stat4 is important for transcriptional activation, although the site of phosphorylation has not been mapped (130). The kinase that mediates the phosphorylation has not yet been identified, but the motif surrounding serine 727 suggests that it will be an enzyme with a specificity similar to that of MAP kinases (131). Stat5 is also reportedly a target for serine phosphorylation, but it lacks the putative MAPK phosphorylation site (132). For STATs containing a serine that is phosphorylated, it is unclear whether this residue alone is important or whether other sequences also contribute to the transcriptional activation domain. Interestingly, Stat2 contains an acidic C-terminal transcriptional activation domain but Stat2 is not a target for serine phosphorylation (126). Thus, it is clear that serine phosphorylation is not the only important feature of the C-terminal region. Indeed, as noted below, the C-terminal region of Stat2 can bind the p300/CBP transactivator proteins (133–135).

In addition to whatever intrinsic transcriptional activation activity the STATs might have, at least some STATs have been demonstrated to associate physically and functionally with coactivator proteins. First, Stat1 has been reported to associate with the transcriptional activator Sp1 in the context of the ICAM-1 promoter, where binding sites for each of these proteins have been found (136). Second, a truncated version of Stat3, denoted Stat3β, was shown to associate with c-Jun; this interaction was discovered by the use of a yeast two-hybrid screen (137). Third, the potent transcriptional activators CBP (cAMP response element binding protein, or CREB binding protein)/p300 have been shown to interact with Stat1 and Stat2 (133–135). Interestingly, p300 interacts with the N-terminal region of Stat1 via its CREB-binding domain (residues 571–687) and to the C-terminal region of Stat1 via the E1A binding region (residues 1680–1891, the region of CBP/p300 capable of binding the E1A protein of adenovirus) (134). Finally, Stat5a reportedly interacts with the glucocorticoid receptor (138). These types of findings provide a basis for the recruitment of transcriptional activators.

In addition to the observation that STAT proteins cooperate with other proteins, it is striking that functional STAT binding sites (GAS motifs) are often found in close proximity to each other, as for example in the promoters for IFNγ (139) and the IL-2 receptor α chain (IL-2Rα promoter) (140–142). It is therefore interesting that the N-terminal region of Stat1, and perhaps the N-termini of other STAT proteins as well, can mediate the dimerization of STAT dimers (139, 143). Thus, a promoter with adjacent GAS motifs can recruit

two STAT dimers which can physically interact, thereby forming a tetrameric structure, and can bind to the promoter with higher affinity. In some cases, one of the GAS motifs is imperfect and a poor STAT binding site (139–141); nevertheless, in conjunction with another site, such imperfect GAS motifs are important. This mechanism of cooperative binding presumably provides the ability to achieve much higher STAT binding affinity and specificity by bringing together the proper dimers. Interestingly, DNA binding activity is not detected to the IL-2 response element in the IL-2Rα promoter when either the consensus or the nonconsensus GAS motif is mutated (141). In this element, it is striking that binding of an Ets family protein, Elf-1, is also vital for promoter activity (140, 141), suggesting a functional cooperation of Elf-1 for Stat5-mediated activation of the IL-2Rα promoter. It is possible that more than one cooperating factor can associate with the same STAT, providing a mechanism by which that same STAT can be involved in the activation of different genes (with binding sites for different cooperating factors) in different cell types or activation states, depending on which cooperating factor is expressed.

CONTRIBUTION OF STATS TO SPECIFICITY OF CYTOKINE SIGNALING

All interferons and type I cytokines can activate one or more STATs. Given only seven known STATs and so many cytokines, it is clear that each cytokine cannot have its own STAT. Nevertheless, it is interesting that cytokines fall into groups and there is considerable specificity to the various STATs (see Table 1). This has been confirmed by the analysis of mice in which various STATs have been deleted by homologous recombination (Table 2). For example, Stat1-deficient mice exhibit a selective defect signaling in response to both type I and type II IFNs (144, 145). Although Stat1 has been reported to be activated by a number of other cytokines and growth factors, including growth hormone, IL-2, EGF, etc, the phenotype of the Stat1-knockout mice indicates either that the role of Stat1 for other cytokines is not physiologically important, or that Stat1 plays roles in functions redundantly served by other STATs or non-STAT proteins. Although we cannot yet distinguish between these possibilities, it may be relevant that many reports of the activation of STATs by certain cytokines/growth factors have used cell lines rather than primary cells and have used extremely high concentrations of the cytokines/growth factors, suggesting the need for caution in interpreting these types of experiments.

Corresponding to the role of IL-12 and IL-4 in Th1 and Th2 cell development, mice lacking Stat4 (146, 147) or Stat6 (148–150) exhibit defective Th1 or Th2 cell development, respectively. Mice lacking expression of Stat5a exhibit defective lobulo-alveolar development and milk production in the mammary

gland, consistent with the importance of Stat5a for prolactin signaling (151), whereas mice lacking Stat5b exhibit defective growth similar to that found in Laron-type dwarfism, a disease resulting from defects in the growth hormone receptor (179). Mice lacking Stat5a have also been shown to have lymphohematopoietic defects (180, 181). Bone marrow–derived macrophages from Stat5a-deficient mice exhibit defective responsiveness to GM-CSF (180), and Stat5a-deficient T cells exhibit defective IL-2-induced receptor α chain expression (180), consistent with the Stat5 dependence of the IL-2Rα IL-2 response element described above. It is interesting that murine embryos lacking Stat3 can implant, but fetal growth and development are then greatly compromised, resulting in very early death (149), earlier than is seen in mice lacking gp130, indicating another role for Stat3 in addition to its activation in response to the IL-6 family of cytokines.

Overall, the effects that have been seen in the various knockout mice are consistent with very selective defects in signaling by different cytokines. Thus, STATs seem to provide important selectivity. Nevertheless it is clear that STATs activated by many cytokines (e.g., Stat3, Stat5a, and Stat5b) cannot by themselves determine the unique actions of a diverse set of cytokines; specificity may depend on these STATs acting in conjunction with the proper combination of other transcription factors and signaling molecules.

STATS IN PROLIFERATION AND TRANSFORMATION

Considerable controversy has surrounded the range of biological actions of STATs including their roles in mediating proliferative responses. IFNs are antiproliferative, and signaling is associated with differentiation to achieve the antiviral state. As a result, Stat1 and Stat2 have been assumed to play a role related to differentiation. However, most of the type I cytokines are mitogenic, so it would make sense that some STATs may contribute directly or indirectly to mitogenic responses (1, 4). Indeed, a variety of data support this hypothesis. First, in some biological systems STAT proteins are constitutively activated following viral or oncogene-mediated transformation; this has been observed following infection of cells with human T-cell lymphotropic virus, type I (HTLV-I) (98), v-Abl (100), *raf/myc* or *abl/myc* viruses (152), spleen focus forming virus (153), and v-Src (154). Second, for some receptors such as IL-2 receptor β chain, mutagenesis of the tyrosines whose phosphorylation creates docking sites for Stat5 results in diminished proliferation (122, 155). In the case of the erythropoietin receptor, one group concluded that mutation of Stat5 docking sites had no effect on proliferation (156), but another group found a significant effect (157). Third, a dominant negative Stat5 construct significantly inhibited IL-3-induced proliferation (158). Fourth, mice lacking Stat4 and Stat6 expression exhibit diminished proliferation in response to IL-12

and IL-4, respectively (146–150). Finally, studies in *Drosophila* also support a role of STATs in mediating proliferation (94). Specifically, a reduction in the amount of *STAT92E* gene activity suppressed the transforming ability of $hop^{Tum\text{-}l}$, a gain-of-function Jak mutation (159–160). Thus, there are compelling data to support a role for STATs in proliferation. However, it is important to recognize that the role may not be direct. For example, in the case of the Stat6 knockout mouse, expression of the IL-4 receptor α chain is diminished (150), consistent with a role for IL-4 and Stat6 in regulating IL-4Rα expression, making it impossible to determine whether the effect on proliferation was "direct" or instead a result of diminished receptor numbers. In Stat5a-deficient mice, there is defective IL-2-induced proliferation at standard levels of IL-2. However, this results from defective IL-2-induced IL-2Rα expression (and hence diminished high-affinity receptors) rather than an intrinsic defect in proliferation, because maximal proliferation is still achieved at concentrations of IL-2 sufficient to titrate the IL-2Rβ-γ_c intermediate-affinity IL-2 receptors (181).

In contrast to mitogenic cytokines, IFNs can exert antiproliferative actions. It is therefore interesting that Stat1 can induce the expression of $p21^{WAF1}$ (161), a cyclin-dependent kinase (cdk) inhibitor. Indeed, this role for Stat1 may be of pathophysiological relevance in thanatrophoric dysplasia type II dwarfism (162).

OTHER ROLES FOR STATS

In addition to their role in modulating transcription, another type of role for STATs has been suggested by the ability of Stat3 to serve as a mechanism for recruitment of PI 3-K to the IFNAR-I component of type I IFN receptors (163). Thus, the existence of a receptor-associated STAT protein could represent a basis for the recruitment of other signaling molecules. Stat1 also mediates constitutive expression of caspases apparently independent of interferons and Stat1 dimerization (184).

NEGATIVE REGULATION OF STATS

The activation of STATs has been described above. How are STATs turned off? A number of different potential mechanisms exist. First, dephosphorylation of the critical tyrosine in the vicinity of amino acid 700 of STATs would result in the loss of dimerization and inactivation of DNA binding. Indeed, it has been reported that this is a mechanism (164). For STATs where serine phosphorylation is vital for action, dephosphorylation of a regulatory serine (e.g., serine 727 of Stat1 or Stat3) would also shut off a signal. In this regard, the N-terminal region of Stat1 has been implicated in the binding of a phosphatase (165), and mutation of this region can lead to constitutive activation. Second, degradation of STATs

would be a logical mechanism. In this regard, it has been demonstrated that Stat1 is a target of ubiquitin/proteasome-mediated degradation (166). Stat1 is also known to undergo alternative splicing, resulting in both 84- and 91-kDa forms of Stat1, where only the longer form is active (167). Stat5 proteins are well known to exist in multiple forms, resulting from degradation (168) and alternative splicing (116, 169). Interestingly, one alternatively spliced form of Stat5b is missing much of the DNA binding domain (116), whereas another form, analogous to Stat1, is missing the C-terminal region (169). Third, the CIS/SOCS/JAB/SSSI family of proteins can inhibit STAT-dependent signaling (78–81, 182). Thus, in addition to direct inactivation of STATs by dephosphorylation or degradation, these other proteins have the ability to serve in a more classical negative feedback loop to regulate cytokine signaling. Finally, there is now an example of another class of protein that can compete for a STAT binding site. Specifically, BCL-6 is able to compete with Stat6 for binding to target sites, thus perhaps explaining the important role played by BCL-6 in germinal center formation (170).

GENES ACTIVATED BY STATS

STATs were originally discovered based on studies of IFN-inducible genes (6). Thus, a potential family of STAT-regulated genes was known even before STATs were well studied, and much is known about the roles of IFNα/β-induced Stat1-Stat2-p48 complexes and IFNγ-induced Stat1 homodimers. However, for the four-helical bundle type I cytokines, much less is known regarding the range of genes activated by induced STAT complexes. Stat5a was originally discovered in the context of prolactin signaling as a mammary gland factor. As a result, it was assumed to regulate the β-casein gene. However, in the Stat5a knockout mice, β-casein expression is essentially unaffected (151), whereas expression of the whey acidic protein (WAP) gene is clearly regulated by Stat5a. Other genes known to be regulated by Stat5 proteins include the CIS (171), oncostatin M (172), and IL-2Rα genes (140–142, 173, 181). Stat6 regulated genes include MHC class II, CD23, and IL-4Rα. There is still much to learn regarding the range of genes controlled by STATs, including whether particular genes are regulated by particular STAT homodimers or heterodimers, or whether more than one STAT complex is capable of regulating particular genes. Even for those genes analyzed, the degree of redundancy of different STAT complexes generally remains unclear.

STATS IN EVOLUTION

The basic mechanism of activation of STATs is extremely appealing as a rapid means of signaling from the membrane to the nucleus. As such, it is not

surprising that this mechanism is a very old one. Indeed, Drosophila have a STAT, denoted D-STAT or STAT92E, indicating the role in invertebrates (159, 160, 174). Moreover, a STAT-like protein has now been discovered in *Dictyostelium* (44). This protein has a related DNA binding site, and its activation is based on an SH2 domain. This indicates that the use of tyrosine phosphorylation as a mechanism of activating transcription factors is quite old from a phylogenetic perspective.

OTHER TYPES OF CYTOPLASMIC-TO-NUCLEAR SIGNALING

Two other examples of rapid cytoplasmic-to-nuclear signaling exist, namely the induction of NF-κB (nuclear factor-κB, which is induced by many agents, including certain cytokines, and can regulate the expression of a broad range of genes) (175), and NF-AT (nuclear factor of activated T cells, which is activated by T cell receptor signaling) (176, 177). Whereas activation of a STAT requires its own tyrosine phosphorylation, NF-κB activation involves either serine phosphorylation or ubiquitin-mediated degradation of the inhibitory molecule, IκB (178), and NF-AT activation involves calcineurin-mediated dephosphorylation of NF-AT family proteins. Thus, these three systems each act in a different context, but each uses a phosphorylation-based level of control to effect rapid cytoplasmic-to-nuclear translocation.

CONCLUSIONS

STATs and Jaks together constitute a signaling system of tremendous importance about which much information has been gathered in the past few years. However, much remains to be learned. The regulation of the Jaks and the function of the various JH domains need clarification. In addition, it will be important to clarify the range of substrates, beyond STATs, that are targets of Jak family kinases. Solution of the three-dimensional structure of the Jaks and STATs is eagerly anticipated. Moreover, as indicated above, the negative regulation of both the Jaks and STATs is an area in need of further clarification, both in terms of the roles of phosphatases and regulated degradation. The region between the DNA binding domain and SH2 domain, although originally suggested to have homology to SH3 domains, in fact, has not been demonstrated to bind proline-rich regions. Nevertheless, this region and others are conserved, suggesting that they may have important functions that still remain to be elucidated. The range of genes activated by STATs and the protein-protein interactions that mediate these phenomena are extremely important areas for future studies. A major challenge that remains is to understand the mechanisms by which specificity in signaling is achieved. Presently, the STATs have provided a number of

clues as to how this occurs, but transcriptional regulation of genes typically is achieved by the coordinated effect of multiple factors, making it necessary to understand how STATs integrate into the overall program of gene activation.

Finally, it is clear that cytokines can also activate many other pathways, including those that couple to Ras/Raf/MAPK, insulin receptor substrate-1 and -2, phosphatidylinositol 3-kinase, Akt, and p70 S6 kinase, to name a few. It is the cytokine-specific interaction of multiple pathways that ultimately integrates a complex array of signals into specific cellular functions such as proliferation, differentiation, and the inhibition of apoptosis. Understanding the contribution of Jaks and STATs to these pathways and the crosstalk among the pathways will be essential to our understanding of the molecular basis of cytokine action.

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