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The Janus kinase family of protein tyrosine kinases and their role in signaling

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Abstract. In the early 1990s, the search for protein kinases led to the discovery of a novel family of non-receptor tyrosine kinases, the Janus kinases or JAKs. These proteins were unusual because they contained two kinase homology domains and no other known signaling modules. It soon became clear that these were not 'just another' type of kinase. Their ability to complement mutant cells insensitive to interferons and to be activated by a variety of cytokines demonstrated their central signaling function. Now, as we approach the end of the decade, it is evident from biochemical studies to knockout mice that JAKs play non-redundant functions in development, differentiation, and host defense mechanisms. Here, recent progress is reviewed, with particular emphasis on structure-function studies aimed at revealing how this family of tyrosine kinases is regulated.

Key words. JAK; protein tyrosine kinase; cytokine; receptor; signaling; STAT.

Introduction

There are presently four identified mammalian Janus kinase (JAK) family members: JAK1, JAK2, JAK3, and TYK2, which range from 120 to 140 kDa. The history of their identification has been recently reviewed [1, 2]. While JAK3 is found primarily in hematopoietic cells, the other three members are expressed widely. JAK cDNAs have been isolated from numerous vertebrate species including human, mouse, rat, chicken, and fish. Only one JAK homologue in *Drosophila*, called Hopscotch, has been found [3]. Recently, a secreted protein, named 'unpaired,' which associates with the extracellular matrix, was shown to activate Hopscotch [4]. Although dissimilar to any existing mammalian cytokine, despite the divergence of humans and flies, underlying signaling mechanisms have been conserved in evolution. Moreover, homologues of JAK signaling partners called signal transducers and activators of transcription (STATs; see below) have been identified in *Dictyostelium* [5] and *Caenorhabditis elegans* [6] suggesting the possible existence of JAKs at an even lower end of the evolutionary tree.

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JAKs and their role in signaling pathways

JAKs are activated by soluble intercellular mediators, commonly referred to as cytokines, which share a common α-helical structure and utilize a group of structurally related receptors [7]. The cytokine receptor superfamily comprises polypeptides with a single transmembrane domain and common extracellular structural motifs that are important for ligand binding [8, 9]. Since they possess no intrinsic catalytic activity, they rely on JAKs, which are constitutively associated with their cytoplasmic regions, to transduce the extracellular ligand-binding event to an intracellular signal. Motifs called 'box 1,' usually proline rich, and 'box 2' in the membrane proximal regions of the receptors are important for JAK association. Activation of JAKs occurs within minutes of ligand binding. These kinases are brought together as a result of ligand-induced dimerization/oligomerization of the receptor subunits or, as recently reported, ligand-induced conformational changes of pre-existing dimers [10, 11]. In cases of homodimeric receptors, e.g., for growth hormone (GH), prolactin, thrombopoietin (TPO), and erythropoietin (EPO), only JAK2 is activated. In contrast, heteromeric receptors, made up of different subunits, activate distinct combinations of JAKs. Once activated, JAKs pro-

ceed to phosphorylate the receptor subunit(s) as well as other substrates. STAT proteins are the most studied substrates of JAKs and are recruited into the proximity of the activated JAKs by binding, through their SH2 domains, to phosphotyrosine-based motifs on the receptors. Direct STAT recruitment onto the JAKs themselves has been suggested (see below), although, in contrast to many receptors, specific tyrosine residues have yet to be identified. After being phosphorylated, STATs are able to form homo- and/or heterodimers and translocate into the nucleus, where they act as transcription activators. The combination of activated STATs and other transcription factors contributes to the various cellular responses to different ligands [reviewed in refs 12–15].

In addition to playing a primary role in cytokine signaling, JAKs may also play accessory roles in other pathways. Rapid phosphorylation and activation of JAKs have been reported after stimulation with colony-stimulating factor (CSF)-1 [16], epidermal growth factor (EGF) [17], platelet-derived growth factor (PDGF) [18], insulin, and insulin-like growth factor (IGF)-1 [19], growth factors whose receptors are known to possess intrinsic ligand-inducible tyrosine kinase activity. JAKs also appear to be involved in signaling by G-protein-coupled receptors, including those for angiotensin [20], serotonin [21], and α-melanocyte-stimulating hormone [22]. Recently, JAK2 was reported to interact directly with the cytoplasmic tail of the angiotensin receptor [23]. The JAK/STAT pathway can also be activated in response to tumor necrosis factor (TNF) [24], osmotic shock [25], and upon ligation of MHC-I [26] and CD40 proteins [27].

Structural and functional dissection of the JAKs

JAKs can be roughly divided into an amino-terminal region (N), followed by a catalytically inactive kinase-like (KL) domain and a tyrosine kinase (TK) domain (fig. 1). As mentioned earlier, the presence of an addi-

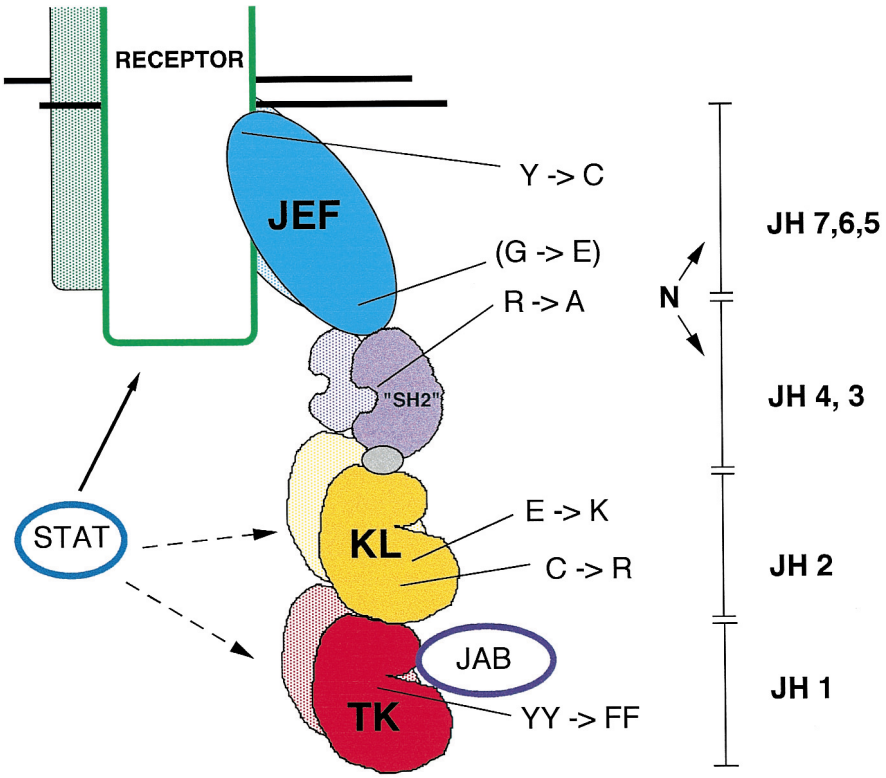

Figure 1. Domain organization of a JAK protein. Two juxtaposed JAK proteins are associated with the membrane-proximal regions of a homodimeric-type cytokine receptor, in a hypothetical 'relaxed' conformation. From top to bottom: the recently proposed band 4.1/JEF domain, corresponding to the JH7-6-5 and part of JH4 boxes (indicated as a vertical line on the right); the potential SH2-related domain, corresponding to parts of JH4 and JH3; a hinge region, in gray, corresponding to the last part of JH3; the kinase-like or KL domain and the tyrosine kinase or TK domain. Mutations discussed in the text are indicated. These are: the JAK3 Y100 to C mutation identified in a SCID patient [36, 45]; the Hopscotch G341 to E [46, 47]; the conserved R of the putative SH2-related domain, mutated in [37]; the Hopscotch E695 to K [57]; the JAK3 C759 to R mutation identified in a SCID patient [58]; the YY in the activation loop of the TK domain (see text). All residues are conserved in the JAK family, with the exception of the Hopscotch-specific G341. STAT proteins interact with receptors and JAKs, although the region of interaction for the latter is unclear. JAB, a member of the CIS/JAB/SOCS/SS1 family, is believed to interact with and inhibit the TK domain.

tional kinase-related domain distinguishes this family of kinases. Interestingly, comparison of intron/exon junctions from the KL and TK domains argues against a simple evolutionary duplication event [28, 29]. Intron/exon patterns for KL are most similar to those of Src family kinases, while those for TK appear to be unique. Alignment of the JAK sequences reveals seven regions of homology, named JH1 to JH7, from the carboxy to the amino terminus. JH3–JH7 correspond to the N region, whereas JH2 and JH1 correspond to the KL and TK domains, respectively (fig. 1). The N region is important for receptor recognition and association. The TK domain is responsible for catalytic activity. The KL domain likely plays a regulatory role, which is not yet well-defined.

The N region

The amino-terminal or N region of the JAKs extends for about 550 amino acids, spanning half of the protein. Determinants of binding specificity to cytokine receptors are located within this region. Interaction studies using JAK segments, expressed in bacteria or in mammalian cells, have contributed to functionally define a critical element spanning approximately the first 200 residues of the JAKs (JH7 and part of JH6) and forming a minimal interaction surface possessing cytokine receptor binding specificity [30–36]. In addition, chimeric JAK constructs in which segments of N were exchanged between two JAK family members have been studied for their ability to reconstitute cytokine-induced responses in JAK-deficient cells [35, 37]. Thus, it was shown that the JH7-6 segment of JAK2 fused to JAK1 was sufficient for binding to the R2 subunit of the interferon (IFN)-γ receptor [37].

On the other hand, this minimal interaction domain was insufficient in other receptor/kinase contexts. The entire N region of JAK1 fused to JAK2 was required for signaling in response to IFN-γ [37]. In a study of the JAK3 interaction with the γ common (γc) receptor chain, it was shown that, although the amino-terminal 193 residues (JH7 and part of JH6) of JAK3 could bind the γc in the COS expression system, 60 additional residues of JAK3 were needed in a JAK3/JAK2 chimera to reconstitute interleukin (IL)-2 signaling [33, 36]. In TYK2, it was shown that the amino terminal 221 residues expressed in vitro interacted efficiently with the IFNAR1 subunit of the IFN-α/β receptor, but additional JH regions were necessary for the in vivo assembly of TYK2 and IFNAR1, through stabilization of IFNAR1 [34, 35].

Thus, the functional and structural organization of the large amino-terminal moiety of the JAK proteins is far from being understood. Intriguingly, sequences corresponding to part of JH3 and JH4 possess structural features of an SH2-like domain [38, 39], although its function has yet to be demonstrated. The conserved arginine of this potential SH2 domain has been mutated into an alanine in JAK1, with no detectable consequences on the IFN-γ signaling capacity of the protein [37].

Some novel insights may come from the recent report that a band 4.1 domain, renamed JEF (see below), is present within the N terminus of JAKs [40]. The band 4.1 domain was originally described in the band 4.1 protein from erythrocytes as a 30-kDa basic charged globular domain which is resistant to mild proteolysis. This protein cross-links the actin cytoskeleton to the erythrocyte membrane through a C-terminal actin-binding domain and the band 4.1 domain. The latter interacts with the cytoplasmic tails of membrane proteins, such as glycophorins A, C, and band 3 [41]. Quite a diverse group of proteins have been found to contain band-4.1-related domains. Among these are the ERM (ezrin, radixin, moesin) proteins, which also link the actin cytoskeleton to the plasma membrane, the cytoskeletal protein talin, cytoplasmic tyrosine phosphatases, the tumor suppressor schwannomin/merlin, and some motor proteins, like the unconventional myosins [42, 43]. Two related focal adhesion kinases (FAK and Pyk2) and the JAKs have been recently added to the list. Consequently, it has been referred to as the band 4.1/JEF (JAK/ERM/FAK) domain [44]. In their study, Girault et al. [44] used, among other approaches, a bidimensional method of sequence analysis (hydrophobic cluster analysis) which combines sequence comparison and secondary-structure prediction, allowing detection of similar hydrophobic clusters within domains of very low (15–20%) sequence identity. Their analysis suggests that the band 4.1/JEF domain may consist of a duplicated module, resulting from an old evolutionary event.

In the JAK proteins, the band 4.1/JEF domain extends for about 450 residues, from JH7 to half of JH4 (fig. 1), with intervening sequences of variable lengths between conserved blocks of hydrophobic residues [44]. Although more work is needed to confirm the presence of such a domain and its functional implications, a number of indirect observations support its existence. It was found that the single Y100 to C substitution present in a mutant JAK3 allele from a severe combined immunodeficiency (SCID) patient [45] abolished the interaction of the kinase with the γc chain in the IL-2 receptor, whereas the substitutions Y to A or Y to F had no effect [36]. Tyrosine 100 maps to JH7 and, interestingly, in one of the most conserved hydrophobic blocks of the band 4.1/JEF domain. Other point mutations were generated in the region surrounding this

tyrosine and only those in highly conserved band 4.1/JEF residues were disruptive. Another observation supporting the existence of the band 4.1/JEF domain comes from mapping of the minimal domain of TYK2 required for interaction with the IFN-α/β receptor chain IFNAR1 [35]. The amino boundary of this domain maps precisely at the boundary of the band 4.1/JEF domain. Interestingly, an activating gain-of-function mutation (G341 to E) identified in Hopscotch [46, 47] maps in a Hopscotch-specific loop of the band 4.1/JEF domain.

Studies of other members of this band-4.1/JEF-containing superfamily may shed light on possible roles of this domain in JAKs. The band 4.1 domain mediates intramolecular interactions. Binding of the ERMs to their membrane partners requires the release of the 4.1 domain from an intramolecular inhibition [48]. The amino-terminal band 4.1 domain in FAK could be involved in the inhibition of FAK autophosphorylation activity, through intramolecular interaction [44]. The JEF domain could also mediate heterotypic interactions between members of the superfamily. Ligand-induced interaction between JAK2 and FAK [49] and constitutive interaction between JAK3 and Pyk2 [50] have been recently reported, though the domains involved have not been mapped.

The KL domain

Although the KL domain shares strong similarity with kinase domains, it lacks motifs which are critical for catalytic activity. The inactivation of the TK domain by point mutations or deletion gives rise to a mutant protein with no kinase activity, demonstrating that the KL is a pseudokinase domain [31, 51–55]. Phosphorylation sites in KL have yet to be determined. In JAK2, phenylalanine substitution of two tyrosines conserved in all KL domains did not affect IFN-γ-inducible STAT1 activation [37].

In the hope of understanding the role of this domain, the consequences of its removal have been studied in several systems. Although no direct in vitro kinase assays have been reported, the JAK2 ΔKL mutant appears to be catalytically active, based on its phosphorylation state and its ability to transduce some signaling, including receptor phosphorylation [30, 32, 37, 56]. Interestingly, constitutive phosphorylation or downstream signaling was often seen, suggesting that perhaps deletion of the KL domain leads to desensitization of the receptor/JAK complex to the presence of ligand. In contrast, the deletion of the KL domain in TYK2 [31] and Hopscotch [57] resulted in no detectable downstream signaling events. The TYK2 ΔKL protein was shown to lack in vitro kinase activity, despite its constitutive tyrosine phosphorylation in the cell. Furthermore, a mutant JAK3 protein containing a deletion of seven amino acids at the amino end of the KL domain was recently identified in a SCID patient; no IL-2/IL-4-induced JAK3 or IL-2-induced STAT5a phosphorylation was observed [58].

Observations from IFN-α signaling studies have shown that the KL domain itself can play a positive role, independent of the TK domain. Cells that do not express TYK2 cannot bind IFN-α with high affinity. This phenotype can be rescued by the expression of wild-type TYK2 but not of the N region only [31] However, the expression of N + KL partially rescues this phenotype, resulting in high-affinity IFN-α binding and weak IFN-α-inducible gene expression. Clearly, the KL domain has an active role in this context, perhaps by stabilizing a specific receptor/kinase configuration. Curiously, the role of KL may become minimal in the context of a hyperactive TK since expression of dimerized TEL/JAK2 fusion proteins (see below) in IL-3-dependent BaF3 cells resulted in IL-3-independent growth with no observable differences among TEL/TK, TEL/KL + TK, and TEL/partial KL + TK proteins [59].

The hypothesized function of the KL domain as a negative regulatory domain is largely supported by the identification of a Hopscotch allele with an E695 to K mutation in a residue of the KL domain which is conserved in all JAK family members [57]. This protein was found to be hyperactive and responsible for the tumorous leukemia-like phenotype observed in the affected larvae. When COS cells were transiently transfected with JAK2 bearing the same point mutation, along with STAT5, JAK2 phosphorylation and STAT5 activation increased relative to wild-type JAK2. A simple explanation of these observations is that the point mutation disrupts a negative regulatory domain, thereby activating the protein. However, TYK2-negative cells reconstituted with TYK2 containing the E to K mutation showed no difference in TYK2 phosphorylation levels nor an increase in IFN-α-induced gene transcription, when compared to cells expressing wild-type TYK2 (our unpublished observations). A JAK3 mutant from a SCID patient was recently found to carry a C759 to R point mutation in the KL domain [58]. This mutant JAK3 was hyperphosphorylated, but unable to activate STAT5 in response to IL-2. Yet another point mutation in the KL domain gives rise to a hyperphosphorylated protein with reduced signaling capabilities (our unpublished observations). Thus, disruption of the KL domain does not always lead to a gain-of-function phenotype.

Another proposed role of the KL domain is as a binding domain for STAT proteins. This is especially interesting in that several studies have shown ligand-induced STAT activation in the absence of phosphotyrosine recruitment sites on corresponding receptors [60–62],

suggesting that another mechanism could bring STATs near the JAKs. That this could be achieved directly through the JAKs was first suggested when a yeast two-hybrid screen with the JAK1 KL domain identified STAT5 as a binding partner [63]. Since KL alone would not be phosphorylated in yeast, the interaction is believed to be phosphotyrosine independent. However, in another report on JAK2-STAT5 interaction in yeast, when KL was deleted, the interaction still occurred, suggesting that the KL domain is not absolutely required [64]. Mutation of the conserved arginine residue in the STAT5 SH2 domain affected the interaction, suggesting that this particular JAK-STAT interaction may be phosphotyrosine dependent. Another study reported in vitro association between STAT5 and JAK2 only when JAK2 was activated and STAT5 was not phosphorylated [65]. Finally, several studies have shown the co-immunoprecipitation of JAKs and STATs, yet it is unclear whether the interaction is direct or mediated by an intermediary protein, such as a receptor subunit [66, 67]. These studies do raise the interesting possibility that one role of the KL domain is to allow binding of other effector proteins.

The TK domain

Phospho-transfer activity of JAKs relies on the TK domain. As with other tyrosine kinases, maximal stimulation of JAK activity is attained upon the phosphorylation of tyrosine residues located in a putative activation or A loop in the TK domain [68]. Two tyrosines, whose phosphorylation has been documented, are found in this loop in all members of the JAK family [51, 69]. The role of these tyrosines has been assessed through the study of mutants bearing phenylalanine substitutions (fig. 1). The doubly mutated form of TYK2 (Y1054–1055) retains basal kinase activity, but fails to be induced upon IFN-α binding [70]. The functional consequences of individual tyrosine mutations appear to vary among JAKs. JAK2 relies on the phosphorylation of the first tyrosine (Y1007) for maximal kinase activity and for in vivo reconstitution of EPO signaling. Mutation of the second tyrosine (Y1008), in contrast, had no detectable effect [51]. In a study of JAK3 mutants assayed in COS cells, the first tyrosine, Y980, was shown to act as a critical positive regulatory site, and the second, Y981, as a negative regulatory site [69]. In a separate study, the effect of single or double Y to F mutations in JAK1 and JAK3 were compared by testing their ability to autophosphorylate and activate STAT5 [71]. While JAK1 strictly required the first tyrosine, JAK3 appeared less dependent on the phosphorylation state of either tyrosine. Furthermore, the N terminus was shown to influence the catalytic potential of the protein by altering its dependence on the phosphorylated loop. Altogether, these data suggest subtle differences in the sensitivity of each individual JAK to activation by phosphorylation of the activation loop.

A critical question pertains to the identity of the kinase phosphorylating the activation loop. An attractive scenario is that upon cytokine binding, JAKs are *trans*-phosphorylated, as a result of dimerization/oligomerization of the receptor chains, or through conformational changes of preformed receptor complexes [10, 11]. Indirect evidence for such a model comes from the finding that in some heterodimeric-type receptors, kinase-inactive JAKs can be weakly phosphorylated in response to ligand [55, 72]. Although interdependent, different JAKs which are engaged into heterodimeric-type receptors, such as those for IFNs, are not functionally equivalent [54, 55], suggesting that the obligatory position of each enzyme within the receptor/kinase complex could influence its ability to *trans*-phosphorylate and to be phosphorylated.

Finally, it is also conceivable that activation of JAKs could be mediated by other tyrosine kinases such as Src family kinases or receptor tyrosine kinases through direct phosphorylation of the activation loop.

Domain interplay and specificity

Although N, KL, and TK have been discussed separately, these are interdependent modules which need to interact with each other to ensure proper regulation of the protein. Deletions and point mutations in the N region or in the KL domain appear to increase the basal tyrosine phosphorylation and the kinase activity of the mutant protein [53] (discussed above), suggesting that both N and KL contribute to the regulation of TK. The interaction of the N region with the receptor, in addition to properly localizing the protein, could influence the accessibility of the catalytic portion of the protein. Consequently, disruption of this interaction would affect TK regulation and substrate specificity. Through its interaction with the N region, the KL domain can exert an indirect effect on the ligand-binding activity of the receptor [70].

Functional studies of chimeric proteins, in which N regions were exchanged, have offered some insight into the specificity of each domain. TYK2-negative cells can respond normally to IFN-α when they are reconstituted with a chimera containing the N region of TYK2 and the KL/TK domains of JAK1, suggesting that the KL and TK domains are interchangeable [35]. Similarly, JAK1-negative cells can respond to both IFN-α and IFN-γ when they express a chimera containing the N region of JAK1 and the KL/TK domains of JAK2 [37]. These and other observations [73] suggest that the posi-

tioning of the JAK, via its N region, in the receptor/JAK complex is the most important determinant of substrate specificity. Intrinsic properties of the KL and TK domains may, however, also contribute. In the study just discussed, class I HLA expression and protection against EMC virus were restored but not to wild-type levels, suggesting that KL and TK domains do bring in a certain degree of specificity [37]. Consistent with this, some peptide substrate specificity was detected in a study comparing the phosphorylating ability of isolated TK domains from three JAKs [74].

JAK-interacting partners

In addition to binding to receptors, members of the JAK family have been reported to interact with a number of intracellular signaling proteins, including tyrosine phosphatases, protein and lipid kinases, STATs, and adaptor molecules (table 1). Constitutive as well as ligand-induced associations have been described. Most of the proteins listed in table 1 have been shown to undergo tyrosine phosphorylation upon cytokine addition and thus are likely substrates and downstream effectors of JAKs. Interesting partners are those that are able to downregulate JAK function, such as the JAB inhibitor and the protein tyrosine phosphatase SHP-1 (see below). Although the biological implications of most of these interactions remain quite elusive, they reinforce the concept that JAKs are central regulators of many cytokine-induced signaling events and not only of STAT-mediated transcriptional activation.

Table 1. Proteins reported to interact with JAKs.

Partner	JAK	Reference
STAT-5	JAK1, JAK2, JAK3, TYK2	63–65, 67
STAT3	JAK2	66
JAB/SOCS-1/SSI-1	JAK1, JAK2, JAK3, TYK2	77–78
SHP-1	JAK2, TYK2	121–123
SHP-2	JAK1, JAK2	124–127
Raf-1	JAK1, JAK2, TYK2	128, 129
Eyk	JAK1	130
v-Abl	JAK1	118
Fyn	TYK2	131
Tec	JAK1, JAK2	132
Btk	JAK1	132
FAK	JAK2	49
Pyk2	JAK2, JAK3	50, 143
PI3K	JAK1	93
cPLA2	JAK1	133
Shc	JAK2	134–135
Grb2	JAK1, JAK2	136, 142
c-Cbl	TYK2	137
Crk-L	TYK2	138
Vav	TYK2	139
IRS2	TYK2	140
STAM	JAK2, JAK3	141
HBx	JAK1	116

Downregulation of the JAKs

The most interesting development concerning the downmodulation of cytokine signaling has been the discovery of the CIS/JAB/SOCS/SS1 family. Briefly, these are SH2-containing proteins whose expression is induced by cytokines. Several of them have been shown to negatively regulate JAK/STAT pathways, by acting on the receptor or the JAKs themselves [75–81].

Other downregulatory mechanisms can, of course, come into play. Recent results suggest that for cytokine receptors, internalization and signaling are two independent processes. Studies with chimeric receptors containing the extracellular domains of the α- and β-chains of the IL-5 receptor and the intracellular region of gp130 have shown that, in this system, receptor endocytosis is not dependent on an active JAK-STAT pathway [82]. When the box 1 motif was deleted, abolishing JAK2 binding, the rate of receptor internalization was unaffected. In addition, disruption of a dileucine internalization motif in the receptor, which resulted in defective receptor internalization, had no effect on IL-5-induced STAT activation. Thus it appears that receptor endocytosis is not a principal mode of ligand-induced JAK downregulation [83].

Another common mechanism of protein downregulation involves the ubiquitin-proteasome system. Proteins are tagged by the addition of ubiquitin by ubiquitinating enzymes. Proteasome complexes containing proteolytic activity are then able to recognize and degrade these proteins [84]. Although the involvement of proteasomes has been suggested for STAT1 regulation [85], it remains questionable if this is the case for JAKs. In CHO cells which have a temperature-sensitive defect in ubiquitination, GH-induced STAT5 activation, but not JAK2 phosphorylation, was affected at the non-permissive temperature [86]. Several studies have shown that the addition of proteasome inhibitors results in the prolongation of JAK activation [87, 88], but neither direct ubiquitination nor an increase in JAK protein levels has been demonstrated. It has been proposed that the proteasome inhibitor is instead preventing degradation of a regulatory protein, such as a JAK phosphatase inhibitor, resulting in prolonged JAK activation. Interestingly, DUB-1, a deubiquitinating enzyme, was identified as an IL-3-induced gene [89].

Protein tyrosine phosphatases likely play a direct role in the regulation of JAKs. Of two related SH2-containing phosphatases shown to interact with JAKs, SHP-1 and SHP-2 (table 1), SHP-1 appears to play a major role in deactivating receptor/JAK complexes. Study of the

SHP-1-deficient *motheaten* mouse demonstrated the negative role of SHP-1 in signaling through hematopoietic receptor tyrosine kinases, antigen and Fc receptors, as well as cytokine receptors [90]. SHP-1 was shown to be recruited to phosphotyrosine-containing motifs of activated cytokine receptors, probably through an SH2-mediated interaction [91–93]. Substrates of SHP-1 are most likely the receptors and the JAKs. Interestingly, low or no SHP-1 expression was observed in IL-2-independent human T cell lymphotrophic virus (HTLV)-1-transformed T cells, suggesting that downregulation of SHP-1 expression could be a step towards cytokine independence [91–93]. Recently, a role for SHP-2 in attenuating JAK activation mediated by the gp130 receptor was suggested [94].

In addition, the use of inhibitors and drugs have led to observations that hint at potential regulatory mechanisms. GH-induced activation of JAK2 can be prolonged for hours in the presence of cycloheximide, suggesting that ongoing protein synthesis is needed for the attenuation of the JAK phosphorylation signal [95]. In addition, JAK2 activation can also be prolonged in the presence of phospholipase C (PLC) inhibitors, implicating PLC or the modified lipids as players in JAK downregulation [95]. Pretreatment of cells with forskolin, which stimulates adenylate cyclase, can lead to a decrease in IFN-β-induced TYK2 and JAK1 phosphorylation [96]. IL-6-induced JAK1 activity is inhibited in the presence of the calcium ionophore ionomycin [97]. Finally, it was shown that the redox state of JAKs can affect catalytic activity, independently of phosphorylation state [98].

JAK knockout mice

Recently, the study of mutant mice which are deficient in a single JAK and the close inspection of cytokine-induced signaling in their primary cells have provided more insight into the essential and non-redundant roles of JAK family members (table 2).

Table 2. Phenotypes of JAK knockout mice.

JAK1	perinatal death, defect in nursing block in lymphocyte development no response to IL-2, -4, -7, -9, -13, -15 no response to IFNs and IL-10 reduced responses to IL-6 and leukemia inhibitory factor
JAK2	embryonic death around day 12 lack of definitive erythropoiesis reduced response to stem cell factor and CSF-1 no response to EPO, TPO, IL-3, IL-5 no response to IFN-γ
JAK3	viable, fertile, SCID phenotype hypoplastic thymus peripheral T cells present as activated but non-functional developmental pro- to pre-B maturation block lack of natural killer cells, Peyer's patches, fewer lymph nodes
TYK2	viable and fertile (studies in progress)

Newborn mice with homozygous targeted disruption of JAK1 are small and die perinatally from nursing defects [99]. Analysis of primary embryonic fibroblasts and macrophages confirmed the essential role of JAK1 in cellular responses to the IFNs and IL-10. Profound hematopoietic defects were observed in the lymphoid compartment, with deficient thymocyte production and B lymphocyte differentiation. Fetal liver cells from JAK1 $-/-$ embryos failed to proliferate in response to IL-4, IL-2 and, notably, to IL-7. This latter cytokine is known to act early during lymphocyte development. Reduced STAT activation in response to IL-6 or leukemia inhibitory factor (LIF) was measured in different cell types, resulting in defective responses of cardiomyocytes and neurons. This observation suggests that, at least with these cytokines, a threshold level of activated STAT is required to achieve a biological response.

The generation of JAK2-deficient embryos was reported by two groups [100, 101]. Embryos die around day 12 from severe anemia. No definitive erythropoiesis occurs, though erythroid progenitors are present and are rescuable by infection with a JAK2-expressing retrovirus. Fetal liver cell colonies formed in response to granulocyte-colony-stimulating factor (G-CSF), whereas a significant reduction was observed with CSF-1 and stem cell factor. None formed in response to EPO, TPO, or IL-3. Thus, JAK2 appears to play a non-redundant role in signaling through several cytokine receptors. Fetal liver cells from JAK2-deficient embryos could reconstitute peripheral lymphocytes in irradiated JAK3-deficient mice, showing that JAK2 is dispensable for the embryonic development of lymphoid cell lineages. Future conditional gene inactivation and fetal liver rescue experiments will be necessary to define the physiological roles of JAK1 and JAK2 in response to other cytokines and in adult tissues.

JAK3-deficient mice have a phenotype virtually identical to that of γc-deficient mice, with profound defects in the immune system [102–107; and see reviews in refs 108, 109]. Mice have a small thymus with a relatively normal CD4/CD8 thymocyte distribution. Peripheral T cells, although normal in number, have an activated phenotype, express high levels of CD44, and display reduced proliferative capacity in response to IL-2. Furthermore, a defect in negative selection of self-reactive cells has been reported [110]. These results support the

crucial roles of JAK3 in IL-2 signaling and anergy prevention. Contrary to the human phenotype, JAK3 −/− mice have a B cell maturation block in the bone marrow at the pro- to pre-B cell stage. This defect and the reduced cellularity in adult thymus probably result from the absence of response to IL-7. JAK3 knockout mice, like γc knockouts, lack natural killer cells due to the absence of an IL-15 response, and lack $\gamma\delta$ T cells.
Mice homozygously inactivated for TYK2 have been obtained recently (M. Karaghiosoff and M. Müller, personal communication). These animals are viable and fertile, suggesting that TYK2 has no essential role in development. Studies are ongoing to elucidate the physiological role of TYK2 in specific immune responses.

JAKs in pathology

Given their importance in cytokine signaling, it is not surprising that JAKs are involved in immune disorders and disease. SCID is characterized by a block in lymphoid development, which leads to an increased susceptibility to infections. Mutations in the γc receptor chain, or mutations in the associated JAK3, can lead to X-linked or autosomal-linked SCID, respectively [111]. SCID patients with identified mutations in the JAK3 locus either express little or no protein, or non-functional forms [45, 58, 112].
JAKs also play a role in the development of leukemias. In this case, it is not the absence of a functional JAK that leads to disease, but rather its loss of regulation. In three leukemic patients, chromosomal translocations have been identified which resulted in fusion proteins between TEL, a member of the ETS transcription factor family, and JAK2 [113, 114]. These hybrid proteins contain the oligomerization domain of TEL fused to the carboxy-terminal kinase domain of JAK2, resulting in constitutive kinase activity. Transfection of IL-3-dependent BaF3 cells with TEL/JAK2 constructs resulted in constitutive STAT5 activation and IL-3-independent growth [59, 113]. Furthermore, the transplantation of bone marrow retrovirally infected with TEL-JAK2 constructs into mice led to a fatal myelo and T cell lymphoproliferative disorder [59].
Loss of regulation of the JAK/STAT pathway may, in part, explain how certain viral infections can lead to cellular transformation. Infection of IL-2-dependent T cells with HTLV-1 resulted in constitutively activated JAK1, JAK3, STAT3, and STAT5, correlating with IL-2-independent growth [115]. Mouse hepatoma cell lines which stably expressed the HBx protein from the hepatitis B virus had increased activated levels of JAK1, STAT3, and STAT5 [116]. Moreover, HBx was shown to bind to JAK1, which had increased in vitro kinase activity, suggesting a possible mechanism of viral transformation whereby a viral protein could bind and alter JAK activity. Along the same lines, the v-Abl protein from the Abelson murine leukemia virus was shown to interact with JAK1 and JAK3 when transfected into murine pre-B lymphocytes, enhancing JAK kinase activity and leading to ligand-independent STAT activation [117]. Recent studies have mapped the site of JAK interaction to the carboxy end of v-Abl and have shown that this region and a functional JAK1 are necessary for cytokine-independent growth [118]. One possible mechanism is that v-Abl directly binds and activates JAK1 by phosphorylating the tyrosines in its activation loop.
Finally, as more evidence accumulates that an unregulated JAK can lead to dire consequences, two types of *Drosophila* mutants with leukemia-like phenotypes were shown to result from expression of hyperactivated Hopscotch proteins [46, 47, 57].

Conclusions

Many different aspects of the JAKs have been discussed, including their physiological roles, their structure, their regulation, and their role in human disease. Here we mention a few of the many issues which remain to be understood. We are only just beginning to decipher the rules governing the interaction of JAKs with specific cytokine receptors, including the stoichiometry of such complexes. For example, the existence of two JAK-interacting regions within the IL-2 receptor β chain [119] suggests a higher level of complexity. Localization of receptor/JAK complexes, including in specific membrane microdomains, needs to be investigated, as do their potential interactions with membrane lipids and cytoskeletal proteins. Intriguingly, the nuclear localization of JAK1 and JAK2 in CHO cells [120] and of JAK2 in a liver cell line [66] have been reported. Closer inspection of the recently described band 4.1/JEF domain and serious consideration of the existence of a SH2-related domain at the amino terminus of JAK may provide better insight into their mode of activation. Crystallographic structural studies and the identification of tyrosine, serine, and threonine residues that are targets for phosphorylation will certainly provide a wealth of information on JAK regulation. Given the variety of interacting partners identified to date, JAKs could be considered as scaffolding proteins, able to recruit and modulate a variety of signaling molecules. The challenge will be to uncover the specific physiological relevance of each of these interactions. The identification of other point mutations in JAK3 from SCID patients with unique immunologic phenotypes may provide clues to the implication of the JAK/STAT pathway in defined steps of lymphoid development.

Finally, further studies in knockout mice will contribute to our growing knowledge of the JAKs.

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