Protein family review

The Janus kinases (Jaks)

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Summary

The Janus kinase (Jak) family is one of ten recognized families of non-receptor tyrosine kinases. Mammals have four members of this family, Jak1, Jak2, Jak3 and Tyrosine kinase 2 (Tyk2). Birds, fish and insects also have Jaks. Each protein has a kinase domain and a catalytically inactive pseudo-kinase domain, and they each bind cytokine receptors through amino-terminal FERM (Band-4.1, ezrin, radixin, moesin) domains. Upon binding of cytokines to their receptors, Jaks are activated and phosphorylate the receptors, creating docking sites for signaling molecules, especially members of the signal transducer and activator of transcription (Stat) family. Mutations of the *Drosophila* Jak (Hopscotch) have revealed developmental defects, and constitutive activation of Jaks in flies and humans is associated with leukemia-like syndromes. Through the generation of Jak-deficient cell lines and gene-targeted mice, the essential, nonredundant functions of Jaks in cytokine signaling have been established. Importantly, deficiency of Jak3 is the basis of human autosomal recessive severe combined immunodeficiency (SCID); accordingly, a selective Jak3 inhibitor has been developed, forming a new class of immunosuppressive drugs.

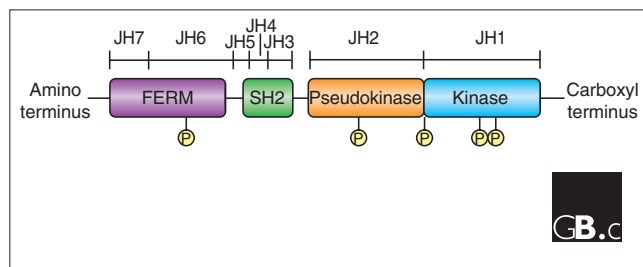## Gene organization and evolutionary history

Janus kinases (Jaks) are non-receptor tyrosine kinases and were discovered in searches for novel protein tyrosine kinases using PCR-based strategies or low-stringency hybridization [1-6]. In mammals, the family has four members, Jak1, Jak2, Jak3 and Tyrosine kinase 2 (Tyk2). In humans, the *Jak1* gene is located on chromosome 1p31.3 and *Jak2* is on 9p24; the *Jak3* and *Tyk2* genes are clustered together on chromosome 19p13.1 and 19p13.2, respectively. The murine genes are located on chromosomes 4 (*Jak1*), 19 (*Jak2*) and 8 (*Jak3* and *Tyk2*). Since the sequencing of other vertebrate genomes has been completed, we know that there are four Jak family members in mammals, birds and fish (see the Additional data files available with the online version of this article for alignments).

Jaks have been identified in the primitive chordate *Ciona*; it is unclear, however, whether this species only has a single Jak or whether more will be found with further sequencing (see Additional data files). In *Drosophila* there is only one Jak kinase, Hopscotch (Hop) [7,8]. The ancestral Jak must therefore have arisen before the divergence of vertebrates and invertebrates. Nematode worms and slime molds lack the family, however, but they do express members of the signal transducer and activator of transcription (Stat) family of transcription factors - which in vertebrates interact with Jaks, among other proteins - suggesting that the Stats arose in evolution before the Jaks. It is of interest that the expansion of the Jak kinases in higher animals occurred at the same time as the evolution of innate and adaptive immune cells in fish; this is consistent with the multiple roles of Jaks

in immune cells (see below). Thus, cytokine receptors acting via Jaks appear to have co-opted the Stat pathway for a variety of purposes, especially for host defense. The proximity of the *Jak3* and *Tyk2* genes suggests that one may have arisen from the other by gene duplication, but it is difficult to conclude which is the more ancestral. Jaks have approximately 20 exons; alternatively spliced forms of Jaks have been described but their functional significance is not known.

Characteristic structural features

The three-dimensional structure of the Jaks is at present unknown. This is no doubt partly because they are relatively large proteins of more than 1,100 amino acids, with apparent molecular masses of 120-140 kDa; expressing and purifying them has been problematic. From the primary structure, putative domain structures have been recognized that are conserved between mammalian, avian, teleost and insect Jaks. Seven Jak homology (JH) domains have been identified, numbered from the carboxyl to the amino terminus (Figure 1). The JH1 domain at the carboxyl terminus has all the features of a typical eukaryotic tyrosine kinase domain. Interestingly, this domain is most closely related to the kinase domains of the epidermal growth factor family of receptor tyrosine kinases, suggesting that the Jak family may have arisen from this larger family of protein kinases [9]. Adjacent to the JH1 domain is a catalytically inactive pseudokinase or kinase-like domain (JH2), which is distantly related to other tyrosine kinase domains [9]. This tandem architecture of kinase domains is the hallmark of Jak kinases and gives them their name; just like the Roman god Janus, they are two-faced with respect to these domains. Although the pseudokinase domain lacks catalytic activity, it has an essential regulatory function. A number of patient-derived and artificial mutations within this domain abrogate kinase activity, underscoring its critical function [10,11]. Conversely, a mutation within

this domain in *Drosophila* Hop activates the kinase and leads to transformation (discussed below) [12-14]. In mammalian Jak2, experimentally introduced mutations in this domain can also increase basal activity, but they abrogate ligand-dependent activation [11,15].

The amino terminus of Jaks contains an SH2-like domain (JH3-JH4) and a Band-4.1, ezrin, radixin, moesin (FERM) homology domain (JH6-JH7). The FERM domain is 300 amino acids long and is implicated in mediating interactions with transmembrane proteins such as cytokine receptors; for some but not all cytokines, Jaks appear to be important in regulating cell-surface expression of the cognate receptors [16,17]. In addition, the FERM domain binds the kinase domain and positively regulates catalytic activity [18]. Unfortunately, the lack of crystal structures severely limits the understanding of the intramolecular interactions that involve Jaks. Binding partners for the Jak SH2 domain have not been identified.

Localization and function

In mammals Jak1, Jak2 and Tyk2 are ubiquitously expressed. In contrast, the expression of Jak3 is more restricted; it is predominantly expressed in hematopoietic cells and is highly regulated with cell development and activation [6,19,20]. At the cellular level, Jaks can be found in the cytosol when they are experimentally expressed in the absence of cytokine receptors, but, because of their intimate association with cytokine receptors, they ordinarily localize to endosomes and the plasma membrane, along with their cognate receptors [21,22]. The link between Jaks and cytokine signaling was first made using mutant cell lines that lacked responsiveness to interferon (IFN). One such cell line was shown to lack Tyk2, and adding back this kinase restored IFN signaling [16]. Shortly thereafter other Jaks were shown to be associated with various cytokine receptors [23-26], and subsequently Jak knockout mice have illustrated their essential and specific functions (see Table 1).

A large number of cytokines are dependent upon Jak1, including a family that use a shared receptor subunit called common γ chain (γc), which includes interleukin (IL)-2, IL-4, IL-7, IL-9, IL-15 and IL-21. These cytokines are also dependent upon Jak3, because Jak3 binds γc. Jak1 is also essential for another family that uses the shared receptor subunit gp130 (IL-6, IL-11, oncostatin M, leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNF)) as well as granulocyte colony-stimulating factor (G-CSF) and IFNs. Jak2 is essential for the hormone-like cytokines such as growth hormone (GH), prolactin (PRL), erythropoietin (EPO), thrombopoietin (TPO) and the family of cytokines that signal through the IL-3 receptor (IL-3, IL-5 and granulocyte-macrophage colony-stimulating factor, GM-CSF). Jak2 is also important for cytokines that use the gp130 receptor and for some IFNs.

Figure 1
A schematic representation of the primary structure of Janus kinases (Jaks), which are made up of FERM, SH2-like, pseudokinase and kinase domains. An alternative nomenclature for the putative domains is as a series of Janus homology (JH) domains. The FERM domain mediates binding to cytokine receptors. Both the FERM and the pseudokinase domains regulate catalytic activity and appear to interact with the kinase domain. Jaks autophosphorylate at multiple sites (P), including two in the activation loop of the kinase domain, but the precise function of these modifications is just beginning to be understood.

Table 1

Functions of Jaks

Gene	Phenotype of mouse knockout	Cytokines whose signaling requires this Jak
Jak1	Viable but early postnatal lethal owing to neurological deficits; SCID	Families of receptor with the shared subunits γc or gp130; IFNs
Jak2	Embryonic lethal owing to a defect of erythropoiesis	IL-3; family of receptors with the shared subunit gp130; IFN-γ; hormone-like cytokines (EPO, GH, PRL, TPO)
Jak3	SCID, viable and fertile	Family of receptor with the shared subunit γc
Tyk2	Viable and fertile; susceptible to parasite infection; resistant to LPS; resistant to collagen-induced arthritis	IL-12; LPS

Abbreviations: EPO, erythropoietin; γc, common γ chain; GH, growth hormone; IFN, interferon; IL, interleukin; LPS, bacterial lipopolysaccharide; PRL, prolactin; SCID, severe combined immunodeficiency; TPO, thrombopoietin.

Tyk2 was the first Jak to be implicated in IFN signaling, but subsequent studies indicate that Tyk2 is essential for IL-12 signaling but not for IFN-α/β signaling or for cytokines that use gp130 [27,28]. *Tyk2*$^{-/-}$ mice also have defective responses to lipopolysaccharide (LPS, a component of the outer membrane of Gram-negative bacteria), but whether this is a direct or indirect effect has not been defined. In particular, a role for Tyk2 in signaling through the Toll receptor, which mediates the response to LPS, has not been established [29,30].

Jak1 knockout mice have a perinatal lethal phenotype, probably related to the neurological defects that prevent them from suckling [30] (Table 1). These mice also have defective lymphoid development and function as a result of defective signaling by cytokines through Jak1. Jak2 deficiency results in embryonic lethality at embryonic day 12.5 as a result of a failure in definitive erythropoiesis [31,32]. Interestingly, Jak3 deficiency was first identified in humans with autosomal recessive severe combined immunodeficiency (SCID) [33,34]. We now know that Jak3 binds to γc and that deficiency of either Jak3 or γc abrogates signaling by the family of cytokines using this receptor subunit. Not surprisingly, this has devastating consequences in terms of immune-cell development and function. Together, mutations in the receptor for IL-7, γc and Jak3 account for two-thirds to three-quarters of cases of SCID [35]. *Jak3*$^{-/-}$ mice were subsequently generated, and they too exhibit SCID but notably do not have non-immune defects [36-38]; this is notable because it suggests that an inhibitor of Jak3 would have restricted effects *in vivo* ([35]; see below).

The Jak/Stat pathway has been extensively studied in *Drosophila* and has been demonstrated to be involved in stem-cell maintenance, ovarian-cell migration and sex determination [13,39]. In development, this pathway is important for embryonic segmentation and larval hematopoiesis as well as for development of the eye, wing, trachea, hindgut and limb [14,40-44]. A gain-of-function mutation in *Hop* has been identified that results in a leukemia-like phenotype

in the affected flies; this is designated tumorous lethal (*Hop*$^{tum-l}$) [12,45,46]. In human leukemias, chromosomal translocations result in fusion proteins of the Tel transcription factor with Jaks. This creates a constitutively active Jak, which has also been documented to be transforming [47,48]. In human cells transformed with T-cell leukemia virus-1, Jak3 and Stat5 are constitutively activated [49]. Constitutive activation of Stats is very common in many other types of tumors, although the mechanisms underlying this activation have yet to be defined.

Jaks are constitutively associated with the membrane-proximal regions of cytokine receptors, although in some cases interaction between the Jak and the receptor is increased upon ligand binding (Figure 2). It has been proposed that ligand binding promotes a conformational change in the receptor, which promotes Jak activation through reciprocal interaction of two juxtapositioned Jak kinases and auto- and/or trans-phosphorylation of tyrosine residues on the activation loop of the Jak kinase domain.

Like other tyrosine kinases, Jaks undergo autophosphorylation, but the importance of this modification in Jak-dependent signaling is not very well understood. Autophosphorylation within the activation loop positively regulates kinase activity; in Jak3, however, phosphorylation in this region can enhance or inhibit catalytic activity, depending upon the site of phosphorylation [50] (Figure 1). Other sites of autophosphorylation have recently been identified. For instance, a conserved residue in Jak2 and Jak3 that resides in the hinge region between JH1 and JH2 is a prominent site of autophosphorylation (Tyr813 in Jak2 and Tyr785 in Jak3) [51]. This site serves to recruit the adapter protein SH2-Bβ, which positively regulates Jak2 activity. Other sites of autophosphorylation in Jak2 include Tyr221 and Tyr570 [52].

Frontiers
Despite intensive studies during the past decade that have generated the model shown in Figure 2, the exact molecular
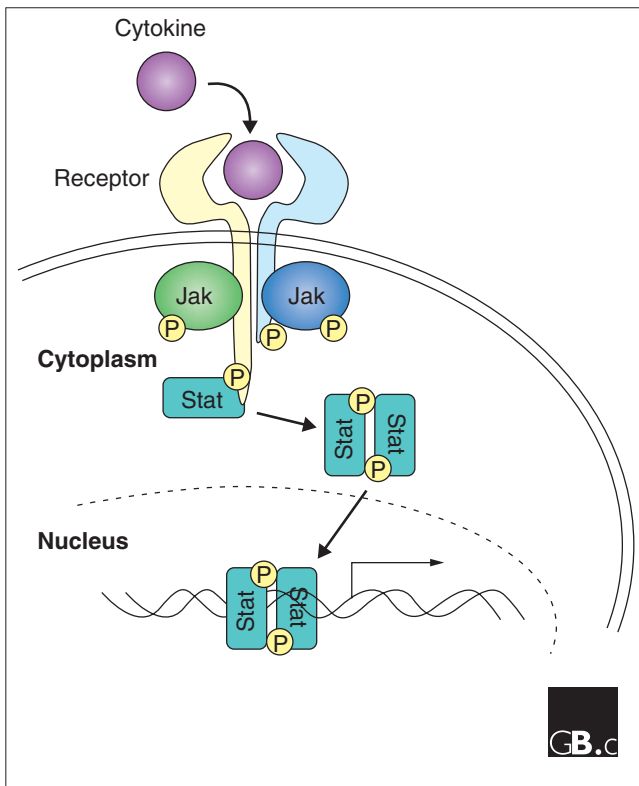
Figure 2
An overview of cytokine signaling. Cytokines bind to homodimeric or heterodimeric receptors, which are constitutively bound to Jaks. Jaks are thought to be activated by a conformational change in the receptor that allows trans- and/or auto-phosphorylation of the two bound Jaks. These in turn phosphorylate the cytokine receptors. Stat proteins bind the phosphorylated receptor chains, allowing the Jaks to phosphorylate the Stats. Phosphorylated Stats form dimers and translocate and accumulate in the nucleus, where they regulate gene expression.

mechanisms of Jak activation have largely remained elusive. It is clear that much more detailed structural information pertaining to Jaks and the Jak-cytokine-receptor complex is needed to enhance our understanding of the mechanism of Jak activation. Also, the exact mechanism and functional relevance of autophosphorylation at different sites in Jaks is not known but will be an interesting area for future research.

Another important topic for future studies is to define the mechanisms of crosstalk between Jaks and other pathways. For instance, the receptor Notch has been reported to promote Stat3 activation, and the Notch effectors Hes1 and Hes5 have been found to associate directly with Jak2 and Stat3 [53]. Evidence for cooperation between the Jak/Stat and Notch pathways has also been provided by work from *Drosophila* [54] and genetic screens in *Drosophila* have identified additional potential modifiers of the Jak/Stat pathway [55]. Jaks have also been reported to be activated by a variety of structurally diverse receptors, beyond the cytokine receptors. Examples include receptor tyrosine

kinases, death receptors (such as CD40) and G-protein-coupled receptors (such as chemokine receptors). Many of the studies have employed overexpression or putatively specific inhibitors to implicate the Jaks, but we now know that these inhibitors are not specific, so the essential function of Jaks for non-cytokine receptors remains uncertain. This is clearly another critical area for future work.

Finally, because of the crucial role of Jak3 in cytokine signaling through γc and because of its limited tissue expression, the inhibition of Jak3 activity has emerged as a promising strategy for immunosuppression. A highly selective and potent Jak3 inhibitor (CP-690,550) has recently been developed that has nanomolar potency against Jak3 *in vitro*, with much less potency against other Jak family members. Consequently, CP-690,550 was both very efficacious and well-tolerated in animal models of organ transplantation [56]. One might anticipate that this drug will help to overcome the unwarranted side effects often seen in patients under current immunosuppressive therapy. Thus, the drug could be useful in blocking transplant rejection and in the treatment of autoimmune diseases. Conceivably, it might also be useful in treating those hematological malignancies that exhibit constitutive Jak3 activation. Targeting Tyk2 with specific drugs would also be logical, given its restricted role; presumably a Tyk2 inhibitor would be useful in some immune-mediated diseases. Whether a Jak2 inhibitor would be useful in malignancies is also worthy of consideration.

Additional data files

Protein sequence alignments in text and jpeg format are available with the online version of this article for orthologs of Jak1 (Additional data files 1 and 6), Jak2 (Additional data files 2 and 7), Jak3 (Additional data files 3 and 8), Tyk2 (Additional data files 4 and 9), and undefined members of the family (Additional data files 5 and 10), and a key for the alignments (Additional data file 11).

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