The JAK/STAT signaling pathway

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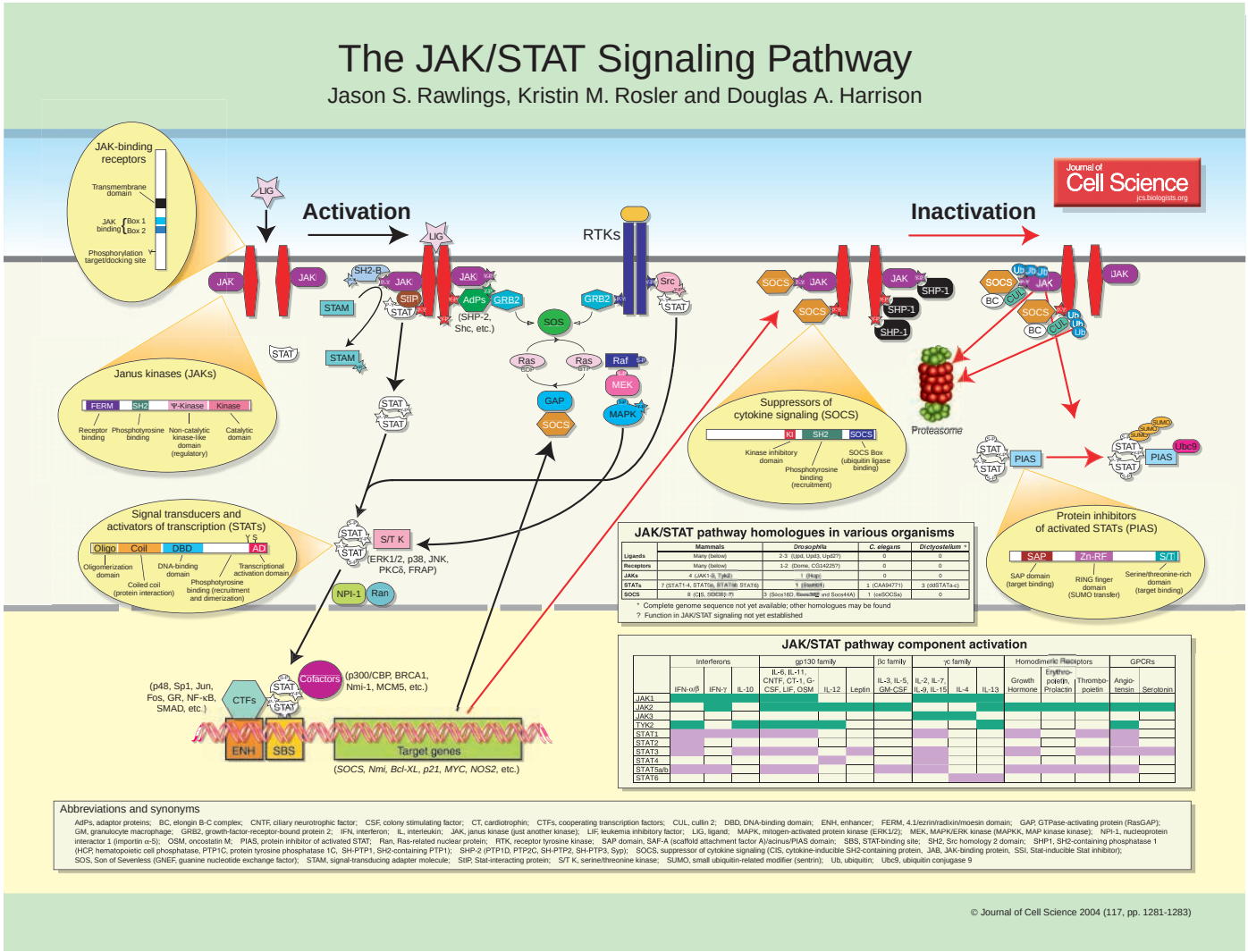
The Janus kinase/signal transducers and activators of transcription (JAK/STAT) pathway is one of a handful of pleiotropic cascades used to transduce a multitude of signals for development and homeostasis in animals, from humans to flies. In mammals, the JAK/STAT pathway is the principal signaling mechanism for a wide array of cytokines and growth factors. JAK activation stimulates cell proliferation, differentiation, cell migration and apoptosis. These cellular events are critical to hematopoiesis, immune development, mammary gland development and lactation, adipogenesis, sexually dimorphic growth and other processes. Predictably, mutations that reduce JAK/STAT pathway activity affect these processes (reviewed by Igaz et al., 2001; O'Shea et al., 2002). Conversely, mutations that constitutively activate or fail to regulate JAK signaling properly cause inflammatory disease, erythrocytosis, gigantism and an array of leukemias. Here we present a general overview of the JAK/STAT pathway and illustrate the primary mechanisms of activation and regulation of this essential signaling cascade.

Mechanistically, JAK/STAT signaling is relatively simple, with only a few principal components (for reviews, see Aaronson and Horvath, 2002; Heinrich et al., 2003; Kisseleva et al., 2002; O'Shea et al., 2002). As described above, a variety of ligands and their receptors stimulate the JAK/STAT pathway. Intracellular activation occurs when ligand binding induces the multimerization of receptor subunits. For some ligands, such as erythropoietin and growth hormone, the receptor subunits are bound as homodimers while, for others, such as interferons and interleukins, the receptor subunits are heteromultimers. For signal propagation through either homodimers or heteromultimers, the cytoplasmic domains of two receptor subunits must be associated with JAK tyrosine kinases. JAKs are distinctive in that they have



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tandem kinase-homologous domains at the C-terminus. The first is a non-catalytic regulatory domain, whereas the second has tyrosine kinase activity. In mammals, the JAK family comprises four members: JAK1, JAK2, JAK 3 and Tyk2. JAK activation occurs upon ligand-mediated receptor multimerization because two JAKs are brought into close proximity, allowing trans-phosphorylation. The activated JAKs subsequently phosphorylate additional targets, including both the receptors and the major substrates, STATs. STATs are latent transcription factors that reside in the cytoplasm until activated. The seven mammalian STATs bear a conserved tyrosine residue near the C-terminus that is phosphorylated by JAKs. This phosphotyrosine permits the dimerization of STATs through interaction with a conserved SH2 domain. Phosphorylated STATs enter the nucleus by a mechanism that is dependent on importin α-5 (also called nucleoprotein interactor 1) and the Ran nuclear import pathway. Once in the nucleus, dimerized STATs bind specific regulatory sequences to activate or repress transcription of target genes. Thus the JAK/STAT cascade provides a direct mechanism to translate an extracellular signal into a transcriptional response.

In addition to the principal components of the pathway, other effector proteins have been identified that contribute to at least a subset of JAK/STAT signaling events. STAMs (signal-transducing adapter molecules) are adapter molecules with conserved VHS and SH3 domains (Lohi and Lehto, 2001). STAM1 and STAM2A can be phosphorylated by JAK1-JAK3 in a manner that is dependent on a third domain present in some STAMs, the ITAM (inducible tyrosine-based activation motif). Through a poorly understood mechanism, the STAMs facilitate the transcriptional activation of specific target genes, including *MYC*. A second adapter that facilitates JAK/STAT pathway activation is StIP (stat-interacting protein), a WD40 protein. StIPs can associate with both JAKs and unphosphorylated STATs, perhaps serving as a scaffold to facilitate the phosphorylation of STATs by JAKs. A third class of adapter with function in JAK/STAT signaling is the SH2B/Lnk/APS family. These proteins contain both pleckstrin homology and SH2 domains and are also substrates for JAK phosphorylation. Both SH2-Bβ and APS associate with JAKs, but the former facilitates JAK/STAT signaling while the latter inhibits it. The degree to which each of these adapter families contributes to JAK/STAT signaling is not yet well understood, but it is clear that various proteins outside the basic pathway machinery influence JAK/STAT signaling.

In addition to JAK/STAT pathway effectors, there are three major classes of negative regulator: SOCS (suppressors of cytokine signaling), PIAS (protein inhibitors of activated stats) and PTPs (protein tyrosine phosphatases) (reviewed by Greenhalgh and Hilton, 2001). Perhaps the simplest are the tyrosine phosphatases, which reverse the activity of the JAKs. The best characterized of these is SHP-1, the product of the mouse *motheaten* gene. SHP-1 contains two SH2 domains and can bind to either phosphorylated JAKs or phosphorylated receptors to facilitate dephosphorylation of these activated signaling molecules. Other tyrosine phosphatases, such as CD45, appear to have a role in regulating JAK/STAT signaling through a subset of receptors.

SOCS proteins are a family of at least eight members containing an SH2 domain and a SOCS box at the C-terminus (reviewed by Alexander, 2002). In addition, a small kinase inhibitory region located N-terminal to the SH2 domain has been identified for SOCS1 and SOCS3. The SOCS complete a simple negative feedback loop in the JAK/STAT circuitry: activated STATs stimulate transcription of the SOCS genes and the resulting SOCS proteins bind phosphorylated JAKs and their receptors to turn off the pathway. The SOCS can affect their negative regulation by three means. First, by binding phosphotyrosines on the receptors, SOCS physically block the recruitment of signal transducers, such as STATs, to the receptor. Second, SOCS proteins can bind directly to JAKs or to the receptors to specifically inhibit JAK kinase activity. Third, SOCS interact with the elongin BC complex and cullin 2, facilitating the ubiquitination of JAKs and, presumably, the receptors. Ubiquitination of these targets decreases their stability by targeting them for proteasomal degradation.

The third class of negative regulator is the PIAS proteins: PIAS1, PIAS3, PIASx and PIASy. These proteins have a Zn-binding RING-finger domain in the central portion, a well-conserved SAP (SAF-A/Acinus/PIAS) domain at the N-terminus, and a less-well-conserved carboxyl domain. The latter domains are involved in target protein binding. The PIAS proteins bind to activated STAT dimers and prevent them from binding DNA. The mechanism by which PIAS proteins act remains unclear. However, PIAS proteins have recently been demonstrated to associate with the E2 conjugase Ubc9 and to have E3 conjugase activity for sumoylation that is mediated by the RING finger domain (reviewed by Jackson, 2001). Although there is evidence that STATs can be modified by sumoylation (Rogers et al., 2003), the function of that modification in negative regulation is not yet known.

Although the mechanism of JAK/STAT signaling is relatively simple in theory, the biological consequences of pathway activation are complicated by interactions with other signaling pathways (reviewed by Heinrich et al., 2003; Rane and Reddy, 2000; Shuai, 2000). An understanding of this cross-talk is only beginning to emerge, but the best characterized interactions of the JAK/STAT pathway are with the receptor tyrosine kinase (RTK)/Ras/MAPK (mitogen-activated protein kinase) pathway. The relationship between these cascades is complex and their paths cross at multiple levels, each enhancing activation of the other. First, activated JAKs can phosphorylate tyrosines on their associated receptors that can serve as docking sites for SH2-containing adapter proteins from other signaling pathways. These include SHP-2 and Shc, which recruit the GRB2 adapter and stimulate the Ras cascade. The same mechanism stimulates other cascades, such as the recruitment and JAK phosphorylation of insulin receptor substrate (IRS) and

p85, which results in the activation of the phosphoinositide 3-kinase (PI3K) pathway [for more on PI3K signaling, see Foster et al. (Foster et al., 2003)]. JAK/STAT signaling also indirectly promotes Ras signaling through the transcriptional activation of SOCS3. SOCS3 binds RasGAP, a negative regulator of Ras signaling, and reduces its activity, thereby promoting activation of the Ras pathway. Reciprocally, RTK pathway activity promotes JAK/STAT signaling by at least two mechanisms. First, the activation of some RTKs, including EGFR and PDGFR, results in the JAK-independent tyrosine phosphorylation of STATs, probably by the Src kinase. Second, RTK/Ras pathway stimulation causes the downstream activation of MAPK. MAPK specifically phosphorylates a serine near the C-terminus of most STATs. While not absolutely necessary for STAT activity, this serine phosphorylation dramatically enhances transcriptional activation by STAT. In addition to RTK and PI3K interactions with JAK/STAT signaling, multiple levels of cross-talk with the TGF-β signaling pathway have been recently reported [for a review of TGF-β, see (Moustakas, 2002)]. Furthermore, the functions of activated STATs can be altered through association with other transcription factors and cofactors that are regulated by other signaling pathways. Thus the integration of input from many signaling pathways must be considered if we are to understand the biological consequences of cytokine stimulation.

The JAK/STAT pathway is ubiquitous amongst vertebrates, but can also be found as an intact pathway in some, but not all, other metazoans. A complete JAK/STAT signaling pathway required for an abundance of biological functions is found in *Drosophila* (for reviews, see Hombria and Brown, 2002; Zeidler et al., 2000). There are single homologues for JAK, STAT and PIAS, as well as three SOCS homologues. Each of these components acts in the same fashion as its mammalian counterpart, although no mutants in the fly *SOCS* genes are yet available. Furthermore, there is a single JAK/STAT pathway receptor, Domeless, that shows weak similarities to the cytokine-binding modules of the vertebrate interleukin 6 (IL-6) receptor family. A predicted gene, *CG14225*, encodes a putative protein with similarity to Domeless, but no functional analyses have been reported. Only members of the Unpaired (Upd) family of secreted ligands, Upd and Upd3, have been shown to activate the JAK/STAT pathway in flies (Agaisse et al., 2003; Harrison et al., 1998). Activation by a third homologue, Upd2, has yet to be reported. Although the Unpaireds bear no sequence similarity to cytokines, the predicted highly α-helical nature is consistent with an overall structure that could be similar to cytokines. Sequencing of the *Drosophila* genome has uncovered potential homologues of other JAK/STAT pathway molecules, including STAM, StIP and SH2-B/APS/Lnk family adapters. No mutations of these genes are yet available to test their function in JAK/STAT signaling. For some other organisms, such as *C. elegans* and *Dictyostelium*, there is not a complete functional JAK/STAT cassette, but homologues of some JAK/STAT pathway proteins can be found (Dearolf, 1999; Hombria and Brown, 2002). For instance, STAT homologues in *Dictyostelium* are necessary for chemotaxis, prestalk cell movement during differentiation, and repression of stalk-specific genes. However, there are no JAKs in *Dictyostelium*, but rather STAT activation is mediated by G-protein-coupled receptors (GPCRs) (reviewed by Williams, 2000). Although the link is still controversial and the mechanism unclear, there is mounting evidence that GPCRs also signal through STATs in mammals. This potential commonality in mechanism suggests that portions of the JAK/STAT pathway may have arisen even prior to the appearance of metazoans. Genome sequences from additional organisms and mutants in identifiable homologues will greatly aid elucidation of the mechanism of evolution of the JAK/STAT signaling pathway.

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