Cytokines, a large family of extracellular ligands, stimulate biological responses in virtually all cell types. One important subfamily of cytokines, the hematopoietins, evolved largely to regulate host responses to exogenous stresses, especially infection with pathogens. They stimulate these responses by binding to and activating a family of structurally and functionally conserved receptors. As is the case for many types of receptor families, downstream signaling entails tyrosine phosphorylation. However, the activation of the Janus kinases (Jaks) and their downstream signal transducer and activator of transcription (Stat) proteins is both characteristic of and unique to this hematopoietin family. This signaling paradigm first appeared in invertebrates and has continued to grow in complexity through vertebrate and mammalian evolution.

There are about 50 members of the hematopoietin family in mammals, and they transduce their signals through four Jaks and seven Stats. Consistent with their identity as ILs, many of these ligands play a critical role in regulating leukocyte maturation and activity. Others regulate more central aspects of cellular homeostasis, reflecting the role Stats play in invertebrates. This Perspective series will provide an overview of the Jak-Stat pathway (reviewed in more detail in refs. 1–4) and will explore the important role this pathway plays in regulating immune responses and cellular homeostasis in human health and disease. The series will explore human ailments affected by this pathway, including immunodeficiency, asthma, and cancer. It will also examine the role Stats play in regulating innate immunity and the unique role Stat3 plays in regulating cellular homeostasis.

**The Jak-Stat paradigm**

Characterization of the ability of IFN-α to rapidly induce genes led to the discovery of the Jak-Stat pathway. Subsequent studies determined that Jaks and Stats play a critical role in mediating the biological response to the hematopoietin members of the mammalian cytokine family (Table 1). Hematopoietins exert their potent biological effects through the activation of specific receptors, which can be divided into five subgroups based on their structural features and the Stats they activate (Table 1). A number of receptor tyrosine kinase and G protein–coupled receptors have also been shown to activate Stats, but the role of these signals remains more controversial. When hematopoietins bind their dimeric receptors, they undergo a conformational change that brings receptor-associated Jaks into apposition (Figure 1; ref. 5). The JAKs then sequentially activate each other by phosphorylating specific receptor tyrosine motifs. STATS and other signaling molecules that recognize these motifs, typically through their SH2 domains, are recruited to the receptor and are then themselves activated by a JAK-dependent tyrosine phosphorylation event. The recruitment of unique sets of STATs to each receptor subfamily constitutes a critical step in defining the specificity of the subsequent biological response (Table 1).
IFN, IFN family
IL-23
Stat4
Tyk2
Stat3
Leptin
Jak2
CT-1
Jak1, Jak2?
Stat3
G-CSF
Jak1, Jak2?
Stat3
CNTF
Jak1, Jak2?
Stat3
Jak2 Stat1?
IL-10C
Tyk2
Stat3–6?
STATs may be capable of regulating gene expression even in the absence of a classical stimulus (12).

### The Jaks

There are four mammalian members of the Jak family of receptor-associated tyrosine kinases: Jak1, Jak2, Jak3, and Tyk2 (1). They range in size from 120 to 130 kDa and, except for Jak3, are ubiquitously expressed (3). Jaks consist of seven conserved Jak homology (JH) domains (Figure 2). The carboxy-terminal portion of these molecules includes a distinctive pseudokinase domain (JH1) and a tyrosine kinase domain (JH2) (1). The amino-terminal JH domains, JH3–JH7, constitute a FERM (four-point-one, ezrin, radixin, moesin) domain and mediate association with receptors (2).

Although biochemical studies have implicated differing sets of Jaks for each hematopoietin receptor subgroup (Table 1), the analysis of chimeric receptors suggests that Jaks do not significantly contribute to signaling specificity (1, 2). Nonetheless, Jak gene targeting studies have identified characteristic signaling defects. These studies indicate that Jak1 plays an important role in the biological response to members of the IL-6, IL-2, and IFN/IL-10 receptor families (13). Since the Jak1−/− mice exhibit a perinatal lethal phenotype, a more complete analysis awaits adoptive transfer and tissue-specific knockout studies. The Jak2 knockout mice exhibit a mid-gestational (i.e., day 12.5) lethal phenotype, which has been attributed to a block in definitive erythropoiesis (14, 15). Studies with tissues from Jak2−/− fetal livers indicate defects in the responses to Tpo, IL-3, members of the IL-2 family, and IFN-γ, but not to IL-6 or IFN-α. Tyk2 knockout mice do not exhibit the phenotype anticipated on the basis of earlier biochemical and genetic studies. These mice exhibit only relatively subtle defects in IFN-α/β and IL-10 responses, but profound defects in their responses to IL-12 and, unexpectedly, LPS (16, 17).

The most relevant knockout for human disease is that of Jak3, whose product exhibits a tight and relatively exclusive association with the γC, the γ common receptor chain (3). Since this subunit is shared by the receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 (Table 1; ref. 2), it is not surprising that a loss of its

### Table 1

<table>
<thead>
<tr>
<th>Hematopoietin-dependent JAK-STAT signaling.</th>
<th>JAKs</th>
<th>STATS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Single-chain family</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epo</td>
<td>Jak2</td>
<td>Stat5</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Jak2</td>
<td>Stat5</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>Jak2</td>
<td>Stat5</td>
</tr>
<tr>
<td><strong>gp130 family</strong></td>
<td></td>
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<tr>
<td>IL-6</td>
<td>Jak1, Jak32</td>
<td>Stat5, Stat1</td>
</tr>
<tr>
<td>IL-11</td>
<td>Jak1</td>
<td>Stat3, Stat1</td>
</tr>
<tr>
<td>OSM</td>
<td>Jak1, Jak2</td>
<td>Stat3</td>
</tr>
<tr>
<td>LIF</td>
<td>Jak1, Jak2</td>
<td>Stat3, Stat1</td>
</tr>
<tr>
<td>CNTF</td>
<td>Jak1, Jak2</td>
<td>Stat3, Stat1</td>
</tr>
<tr>
<td>NNT-1/BSF-3</td>
<td>Jak1, Jak2</td>
<td>Stat3, Stat1</td>
</tr>
<tr>
<td>G-CSF</td>
<td>Jak1, Jak2</td>
<td>Stat3</td>
</tr>
<tr>
<td>CT-1</td>
<td>Jak1, Jak2</td>
<td>Stat3</td>
</tr>
<tr>
<td>Leptin</td>
<td>Jak2</td>
<td>Stat3</td>
</tr>
<tr>
<td>IL-12</td>
<td>Tyk2, Jak2</td>
<td>Stat4</td>
</tr>
<tr>
<td>IL-23</td>
<td>?</td>
<td>Stat4</td>
</tr>
<tr>
<td><strong>IFN family</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IFN, type I8</td>
<td>Jak1, Tyk2</td>
<td>Stat1, Stat2, Stats 3–6</td>
</tr>
<tr>
<td>IFN-γ, type II</td>
<td>Jak1, Jak2</td>
<td>Stat1, Stat5</td>
</tr>
<tr>
<td>IL-10C</td>
<td>Jak1, Tyk2</td>
<td>Stat3</td>
</tr>
<tr>
<td>IL-19</td>
<td>?</td>
<td>Stat3</td>
</tr>
<tr>
<td>IL-20</td>
<td>?</td>
<td>Stat3</td>
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<tr>
<td>IL-22</td>
<td>?</td>
<td>Stat3, Stat5</td>
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<tr>
<td>IL-24</td>
<td>?</td>
<td>Stat3</td>
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<tr>
<td>FISP</td>
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Hematopoietins transduce their signals through specific sets of JAKs and STATS as indicated. The assignments with the most confidence, based on knockout and biochemical studies, are shown in boldface. TSLP binds to a related but γC-independent receptor. In humans this family consists of 12 IFN-α’s, IFN-β, IFN-ε, and IFN-λ. IL-10 homologue AK155 has not yet been functionally characterized. Epo, erythropoietin; GH, growth hormone; Prl, prolactin; Tpo, thrombopoietin; TSLP, thymic stromal derived lymphopoietin; LIF, leukemia inhibitory factor; CNTF, ciliary neurotrophic factor; NNT-1/BSF-3, novel neurotrophin-1/B cell-stimulating factor-3; CT-1, cardiotoxin-1; FISP, IL-4 induced secreted protein.

Once activated, STATS are released from the receptor and then dimerize through a reciprocal phospho-tyrosine–SH2 domain interaction (“classical dimerization”); refs. 6, 7; Figure 1). Intriguingly, recent evidence indicates that STATS form stable dimers prior to activation, although this appears to be mediated by a different type of interaction (J. Braunstein and C. Schindler, unpublished observation). Only classically activated and dimerized STATs are competent for rapid nuclear translocation and DNA binding. Although structural studies have provided a detailed insight into how STAT dimers bind DNA, the structural motifs that regulate nuclear import are not well understood (2, 6, 7).
function leads to immunodeficiencies in both humans and mice. Likewise, Jak3−/− mice demonstrate severe defects in lymphocyte development and activity (18–20). As discussed by Candotti et al. (this Perspective series; ref. 21), mutations in Jak3 have recently been found to lead to severe combined immunodeficiency disease (SCID) in some patients.

The Stat family

Seven Stats (Stats 1–6, including two Stat5 genes) have been identified in mammals and range in size from 750 to 900 amino acids. The identification of Stat homologues in simpler eukaryotes suggests that this family arose from a single gene (2, 22). Stats in model eukaryotes, like Dictyostelium, Caenorhabditis elegans, and Drosophila, are most closely related to mammalian Stat3 and Stat5 and appear to regulate developmental processes (reviewed in ref. 2). In Drosophila a single Stat transduces signals through a classical Jak-Stat pathway like that outlined in Figure 1, whereas the Stat homologues in Dictyostelium and C. elegans appear to signal through different pathways. The growth in the number of Stat family members appears to have paralleled the increased need for cell-to-cell communications as eukaryotes became more complex (reviewed in ref. 2).

Stats can be divided into five structurally and functionally conserved domains (Figure 2; refs. 6, 7). The amino-terminal half of the protein consists of two relatively poorly characterized domains. The amino-terminal ∼125 amino acids are well conserved, and this portion of these proteins is reported to promote cooperativity in DNA binding and to regulate nuclear translocation (2, 23). The coiled-coil domain (amino acids ∼135 to ∼315) consists of a four-helix bundle that protrudes about 80 Å laterally from the core structure. This domain associates with a number of potentially important regulatory modifiers, including IRF-9 and StIP1 (4, 24). It has also been implicated in nuclear export (S. Bhattacharya and C. Schindler, manuscript submitted for publication).

The domains that constitute the carboxy-terminus are better understood. The DNA-binding domain (DBD; amino acids ∼320 to ∼480) recognizes members of the GAS family of enhancers and (like the upstream coiled-coil domain) appears to regulate nuclear export (6–8, 10). In the case of Stat2, curiously, this conserved DBD is unable to bind DNA directly (see Decker et al.; ref. 9). The adjacent linker domain (amino acids ∼480 to ∼575) is important in assuring the appropriate structure of the DNA-binding motif and also appears to regulate nuclear export in resting cells (S. Bhattacharya and C. Schindler, manuscript submitted for publication). Not surprisingly, the SH2 domain (amino acids ∼575 to ∼680) is the most highly conserved motif and mediates both receptor-specific recruitment and Stat dimerization (2). All of these proteins but Stat2 are known to homodimerize in vivo. Dimerization requires the binding of a phosphorylated tyrosine activation motif (amino acid ∼700) on one STAT subunit to the SH2 domain of the other subunit (2). Finally, the carboxy-terminus carries a transcriptional activation domain (TAD), which is conserved between homologues (e.g., murine and human). However, the carboxy-terminus varies considerably in both length and sequence between different STAT family members. Once again, Stat2 is an exception, since its TAD sequence diverges considerably between the murine and human homologues (2, 25).

Responses to STAT activation

Gene targeting studies have provided important insight into the functionally distinct roles STATs play in mediating biological responses. Consistent with their identification during the purification of the IFN-activated transcription factors (ISGF-3 and GAF), Stat1 and Stat2 knockout mice exhibit defects in their biological response to both types of IFNs (25–27). As discussed by Decker et al. (9), characterization of these mice has provided important insight into the role the IFNs play in regulating both innate and acquired immunity. The Stat3 knockout mouse exhibits an embryonic lethality, highlighting the important role Stat3 plays in the development of several lineages. Tissue-specific knockouts have been developed to circumvent this difficulty and
have revealed important roles for Stat3 in the development of several tissues (see Levy and Lee, this Perspective series; ref. 28). This panoply of functions is reminiscent of the more pervasive roles Stats play in primitive metazoans. Studies with constitutively active mutants of Stat3 suggest a role in regulation of proliferation and oncogenesis. As Bromberg (this Perspective series; ref. 29) notes, this transcription factor — like some of the other Stat family members and upstream signaling molecules — has emerged as a promising target for cancer therapeutics.

The Stat4 and Stat6 knockout mice exhibit specific defects in their response to the cytokines that regulate the polarization of naive Th cells into the Th1 and Th2 subsets. These cells are known to play an important role in regulating immune response. IL-12 and the closely related IL-23, which signal through Stat4, promote Th1 polarization (Table 1; refs. 30, 31). Conversely, IL-4 and IL-13 signal through Stat6 and promote Th2 polarization (Table 1; refs. 32–34). Both T lymphocyte subsets play an important role in regulating the host response, but in each case their effects must be moderated to avoid disease. Exuberant Th1 responses are associated with autoimmunity, whereas exuberant Th2 responses are associated with allergic diseases like asthma (see Pernis and Rothman, this Perspective series; ref. 35).

The phenotypes of Stat5 knockout mice were unexpected in several respects (1, 36–38). First, Stat5a and Stat5b, which are 96% identical, are not fully functionally redundant, as earlier biochemical studies had suggested. Thus, Stat5a-null mice are defective in pro-lactin-dependent mammary development, while Stat5b knockout mice fail to respond effectively to growth hormone. Not surprisingly, the Stat5a/b double knockout mice exhibit even more severe defects in their response to both of these signals. Second, and in contrast to earlier biochemical studies had suggested, the Stat5a/b double knockout mice develop a full complement of hematopoietic lineages. Their defects in response to signaling by a wide range of cytokines — IL-2, IL-3, IL-5, IL-7, GM-CSF, G-CSF, and Tpo — are remarkably subtle (1, 2).

Concluding comments
Hematopoietins play an important role in regulating leukocyte maturation and activity, as well as the host response to infection. They have therefore become an important focus both in clinical studies and as therapeutic targets. Characterization of the ability of one of these hematopoietins, type I IFN, to rapidly induce gene expression led to the identification of a new signaling paradigm, the JAK-STAT pathway. Subsequent studies determined that this pathway played an important role in transducing signals for all other members of the hematopoietin family (Table 1).

Future studies on this pathway and the biological responses it mediates offer the promise of identifying new therapeutic strategies for a number of human ailments. Important areas that are likely to receive attention include identification of STAT target genes and a better understanding of how STATs interact with other transcription factors to achieve gene regulation. The mechanisms by which STAT signals decay are also poorly understood, although some important regulators of this aspect of the pathway have been defined. These include members of the SOCS family of counter-regulatory proteins (39) (some of which were recently highlighted in the JCI’s December 2001 Perspective series on the immuno-neuroendocrine interface), as well as phosphatases and enzymes that mediate covalent modification of STAT proteins (2). Such modifications include not only ubiquitination, which targets some STATs for proteolysis (11), but also serine phosphorylation (40), and potentially acetylation, arginine methylation, and SUMOylation (2, 41), all potential modifications of STAT proteins (2). Such modifications are likely to be targets of new therapeutic strategies for a number of human diseases like asthma (see Pernis and Rothman, this Perspective series on the immuno-neuroendocrine interface), as well as phosphatases and enzymes that mediate covalent modification of STAT proteins (2). Such modifications include not only ubiquitination, which targets some STATs for proteolysis (11), but also serine phosphorylation (40), and potentially acetylation, arginine methylation, and SUMOylation (2, 41), all potential mechanisms for controlling the duration and consequences of STAT signaling.