Seminars in Cell & Developmental Biology 19 (2008) 311–318

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Review

# Inteferons pen the JAK–STAT pathway

Christian Schindler[a,b,*], Courtney Plumlee[c]

[a] Department of Microbiology, Columbia University, New York, NY 10032, USA
[b] Department of Medicine, Columbia University, New York, NY 10032, USA
[c] Department of Biological Sciences, Columbia University, New York, NY 10032, USA

ARTICLE INFO

*Article history:*
Available online 26 August 2008

*Keywords:*
Interferons (IFNs)
Cytokines
JAKs
STATs and signal transduction

ABSTRACT

Characterization of how interferons (IFNs) mediate their biological response led to identification of the JAK–STAT signaling cascade, where JAKs are receptor-associated kinases and STATs the transcription factors they activate. Today, 4 JAKs and 7 STATs are known to transduce pivotal signals for the over 50 members of the four-helix bundle family of cytokines. This review will provide an overview and historical perspective of the JAK–STAT paradigm.



**Contents**

1. Introduction ..... 311
2. Discovery of the JAK–STAT signaling paradigm ..... 312
3. The Janus kinases (JAK) family ..... 312
4. The STAT family of transcription factors ..... 313
   4.1. Stat1 ..... 314
   4.2. Stat2 ..... 314
   4.3. Stat3 ..... 314
   4.4. Stat4 ..... 314
   4.5. Stat5 ..... 314
   4.6. Stat6 ..... 315
5. Regulating STAT activity ..... 315
   5.1. Covalent STAT modifications ..... 315
   5.2. Phosphatases ..... 315
   5.3. Nuclear import–export ..... 315
      5.3.1. The SOCS family ..... 315
6. A bright future ..... 315
   References ..... 315

## 1. Introduction

Interferons (IFNs), founding members of the cytokine family, were first described by Isaacs and Lindenmann more than 50 years ago [1]. Over the subsequent 25 years, these four-helix bundle cytokines were purified to reveal a surprising biochemical diversity [2]. Concomitant developments in cloning technologies provided both the nascent biotechnology industry with one of its first products and revealed that IFNs can be divided into two major families. Type I IFNs, which included fibroblast (a.k.a.—IFN-β) and leukocyte (a.k.a.—IFN-α's) IFNs, was the larger and more pleiotropic family, whereas type II IFN was represented by a single member, immune IFN (a.k.a.—IFN-γ).

The early availability of recombinant IFNs afforded an opportunity to investigate how cytokines mediate their potent biological responses. Initial cDNA expression studies identified a unique set of IFN stimulated genes (ISGs), as well as distinct type I and II

* Corresponding author at: Columbia University, HHSC 1208, 701 West 168th Street, New York, NY 10032, USA. Tel.: +1 212 305 5380; fax: +1 212 543 0063.
*E-mail address:* cws4@columbia.edu (C. Schindler).

1084-9521/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.semcdb.2008.08.010

**Table 1**
JAK–STAT signaling by the four-helix bundle cytokines[‡]

| Ligands | JAKs | STATs |
|---|---|---|
| IFN family | | |
| IFN-I (type I)[†] | **Jak1**, Tyk2 | **Stat1**, **Stat2**, Stats3, Stat4 (Stats5–6) |
| IFN-γ (type II) | **Jak1, Jak2** | **Stat1** |
| IFN-l (IL-28a,b,-29) | Jak1, Tyk2 | **Stat1**, **Stat2**, Stat3 |
| IL-10 | **Jak1, Tyk2** | **Stat3**, Stat1 |
| IL-19 | Jak1, Jak2 | **Stat3**, Stat1 |
| IL-20 | Jak1, Jak2 | **Stat3**, Stat1 |
| IL-22 (IL-TIF) | Jak1, Tyk2 | **Stat3**, Stat1, (Stat5) |
| IL-24 (mda7) | Jak1, Jak2 | **Stat3**, Stat1 |
| IL-26 (AK155) | Jak1, Tyk2 | **Stat3**, Stat1 |
| gp130 family | | |
| IL-6 | **Jak1**, (Jak2) | **Stat3**, Stat1 |
| IL-11 | Jak1 | **Stat3**, Stat1 |
| LIF | **Jak1**, (Jak2) | **Stat3**, Stat1 |
| CNTF | Jak1, (Jak2) | **Stat3**, Stat1 |
| CLC/CLF[§] | Jak1, (Jak2) | **Stat3**, Stat1 |
| NP | Jak1, (Jak2) | Stat3 |
| CT-1 | Jak1, (Jak2) | **Stat3** |
| OSM | Jak1, (Jak2) | **Stat3**, Stat1 |
| IL-31 | Jak1, (Jak2) | Stat3, Stat5, Stat1 |
| G-CSF | Jak1, (Jak2) | **Stat3** |
| Leptin | Jak2 | **Stat3** |
| IL-12 (p35 + p40) | **Tyk2**, Jak2 | **Stat4** |
| IL-23 (p19 + p40) | **Tyk2**, Jak2 | **Stat3**, **Stat4**, Stat1 |
| IL-27[#] (p28 + EBI3) | Jak2 | **Stat1**, **Stat3**, Stat4, (Stat5) |
| IL-35 (p35 + EBI3) | | |
| γC family | | |
| IL-2 | **Jak1**, **Jak3** | **Stat5**, (Stat3) |
| IL-7 | **Jak1**, **Jak3** | **Stat5**, (Stat3) |
| TSLP[e] | | Stat5 |
| IL-9 | Jak1, Jak3 | **Stat5**, Stat3 |
| IL-15 | **Jak1**, **Jak3** | **Stat5**, (Stat3) |
| IL-21 | Jak1, Jak3 | **Stat3**, Stat5, (Stat1) |
| IL-4 | **Jak1**, **Jak3** | **Stat6** |
| IL-13[¶] | Jak1, Jak2 | **Stat6**, (Stat3) |
| IL-3 family | | |
| IL-3 | Jak2 | **Stat5** |
| IL-5 | Jak2 | **Stat5** |
| GM-CSF | Jak2 | **Stat5** |
| Single chain family | | |
| Epo | **Jak2** | **Stat5** |
| GH | **Jak2** | **Stat5**, (Stat3) |
| Prl | **Jak2** | **Stat5** |
| Tpo | **Jak2** | **Stat5** |

Genetic and biochemical studies have determined that four-helix bundle cytokines transduce their signals through specific JAKs and STATs. Assignments with the highest level of confidence are shown in bold. Those with less confidence are shown in plain lettering, and those with the least confidence are shown in brackets.

[†] In humans this family consists of 12 IFN-αs, IFN-β.ω and Limitin.

[§] a.k.a. NNT-1/BSF-3.

[#] IL-30 is the p28 subunit of IL-27.

[¶] Bind to related, but γC independent receptors.

[‡] Several Interleukins are not members of this family (e.g., IL-1, IL-8, IL-14, IL-16, IL-17, IL-18, IL-25, IL-32, IL-33, IL-34).

receptors [2–4]. Characterization of the ability of IFN-α to drive ISG expression led to the identification of Signal transducers and activators of transcription (Stat)-1 and Stat2 [5–7]. Subsequent studies implicated Tyk2 (a Janus kinase; a.k.a., JAK) and tyrosine phosphorylation in STAT-dependent signaling [8–10]. Over the next several years 7 STATs and 4 JAKs were identified, providing important insight into how the ~50 members of the four-helix bundle cytokine family transduce their potent biological responses (see Table 1). Parallel, but more difficult studies on STAT signal decay identified several families of negative regulators, most notably members of the Suppressors of Cytokine Signaling (SOCS) family (see [154,155] this issue; reviewed in Refs. [11–14]).

## 2. Discovery of the JAK–STAT signaling paradigm

Shortly after the isolation of the first ISGs, an IFN-I specific enhancer, the ISRE (IFN Stimulated Response Element; AGTTTN$_3$TTTCC), was identified [4,6,15]. Analysis of IFN-α stimulated nuclear extracts revealed three distinct ISRE binding complexes: IFN-I Stimulated Gene Factor 1 (ISGF-1; a.k.a. IRF-2); ISGF-2 (a.k.a. IRF-1); and ISGF-3, whose activation correlated directly with the expression of immediate early ISGs [6,15,16]. Purification of ISGF-3 led to identification of four component proteins of 113 kDa, 91 kDa, 84 kDa and 48 kDa [6,7,16,17]. The 48 kDa protein (p48), previously shown to be the DNA binding component, was recognized as a member of the IRF (IFN-I response factor) family of transcription factors and subsequently named IRF-9 [18]. The 84 kDa (p84) and 91 kDa (p91) proteins were found to be alternative mRNA splice products of a single gene. They also exhibited significant homology with p113, but not other proteins (see Fig. 1). The term STAT was subsequently coined, with p84 becoming Stat1β, p91 Stat1α and p113 Stat2 [5,7].

Antibodies developed against Stat1 and Stat2 revealed that both proteins were localized to the cytoplasm in resting cells. Upon IFN-α stimulation, however, Stat1 (both isoforms) and Stat2 were rapidly phosphorylated on a single conserved tyrosine. This led to the formation of a stable Stat1:Stat2 heterodimer and nuclear translocation (see Fig. 1; [8]). Exploiting these antibodies, it was determined that IFN-γ stimulated the rapid activation of just Stat1 [19]. Moreover, the IFN-γ stimulated Stat1 homodimers were found to directly bind to a distinct palindromic sequence, the Gamma-IFN activated site (GAS; TTTCCNGGAAA; [20–23]). Consistent with their ability to activate Stat1, type I IFNs (IFN-Is) were also subsequently determined to direct the formation of Stat1 homodimers and drive expression of GAS target genes, albeit more transiently. Ensuing biochemical and structural studies determined that the stable interaction between the phosphotyrosine of one STAT with the SH2 domain of the corresponding STAT was responsible for dimerization [24–27].

During the same period, founding members of the unique JAK family (i.e., Tyk2, Jak1 and Jak2) were identified through tyrosine kinase homology screens (see Fig. 1; [28,29]). Subsequent elegant genetic and biochemical studies linked Tyk2 with the IFN-I response, and then Jak1 and Jak2 with a number of other cytokines, providing important insight into how cytokine receptors stimulate tyrosine phosphorylation [10,30–33]. The identification of four additional STATs and one JAK provided the tools to determine that all ~50 members of the four-helix bundle family of cytokines transduce their biological responses through this pathway (see Table 1 and Fig. 2; [34–43]). Intriguingly, except for the ~20 type I IFNs (and 3 recently identified type III IFN-Is [44,45]), all other members of this family transduce their signals through the simpler JAK–STAT pathway associated with GAS target genes (see Fig. 2; [46]).

## 3. The Janus kinases (JAK) family

The four JAK family members, Jak1, Jak2, Jak3 and Tyk2, range in size from 120 kDa to 140 kDa, and except for Jak3 (leukocyte-JAK [36]), are expressed in most tissues (reviewed in Refs. [46,47]). This kinase family features seven conserved JAK homology (JH) domains (see Fig. 1), notably including a tandem set of carboxy terminal kinase domains, where only JH1 has bona fide catalytic activity (Ki). JH2 is referred to as the pseudo kinase (ΨKi) domain. Reminiscent of other kinases, activation is driven by phosphorylation of critical tyrosines in the inactivation loop, which releases its blockade of the catalytic site. Although no function has been assigned to the SH2-related domain ("SH2"; JH5 and half of JH4),

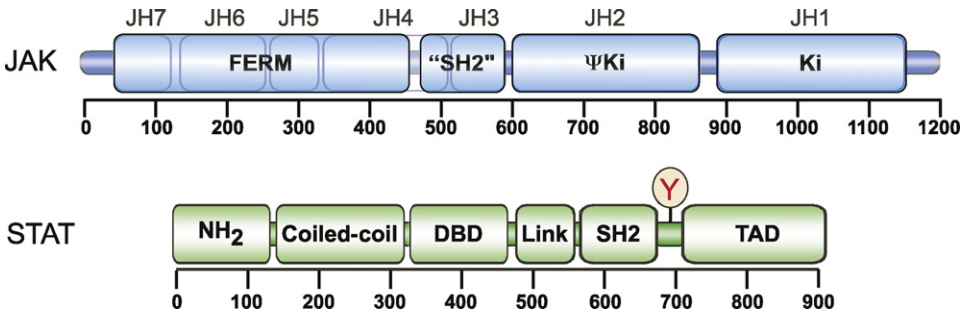


**Fig. 1.** Structural organization of the JAK and STAT families. The *JAKs* share seven JAK homology (JH) domains, JH1–JH7. JH1 serves as the catalytic domain, whereas JH2 represents a pseudokinase domain. JH4 includes an SH2-like domain of unknown function and JH4–JH7 comprise a FERM domain that is responsible for association with cytokine receptors. The six *STATs* share 7 functionally conserved domains. They include the amino terminal domain ($NH_2$), the coiled–coiled domain (Coiled-Coil), the DNA binding domain (DBD), the Linker domain (LK), the SH2 domain, the tyrosine activation domain, and the transcriptional activation domain (TAD).

the amino terminal domains (JH1-3 and half of JH4) constitute a FERM (four point one, ezrin, radixin, moesin) domain, which directs stable association with membrane proximal receptor motifs. As illustrated in Fig. 2, Jak1 stably associates with IFNAR2 and IFNGR1; Jak2 with IFNGR2; and Tyk2 with IFNAR1.

Gene targeting studies have underscored the critical role JAKs play in the biological response to cytokines. Jak1 knockout mice die perinatally due to a failure to nurse (ascribed to a LIF defect). Their tissues are defective in response to cytokines from the IL-2, IL-6, IFN and IL-10 families ([48]; see also Table 1). Jak2 knockout mice exhibit an earlier lethality (i.e., E12.5), reflecting the critical role this kinase plays in definitive erythropoiesis [49,50]. Ex vivo studies on Jak2[−/−] tissues have highlighted the important role this kinase plays in directing signals stimulated by the IFN-γ, as well as the single-chain, IL-2 and IL-3 receptor families (see Table 1). Notably, several myeloproliferative disorders, including the majority of cases of polycythemia vera, essential thrombocythemia and primary myelofibrosis have been attributed to a single Stat5-activating point mutation in the JH2 domain of Jak2, V617F, underscoring a potential therapeutic target (see [51,156–158]).

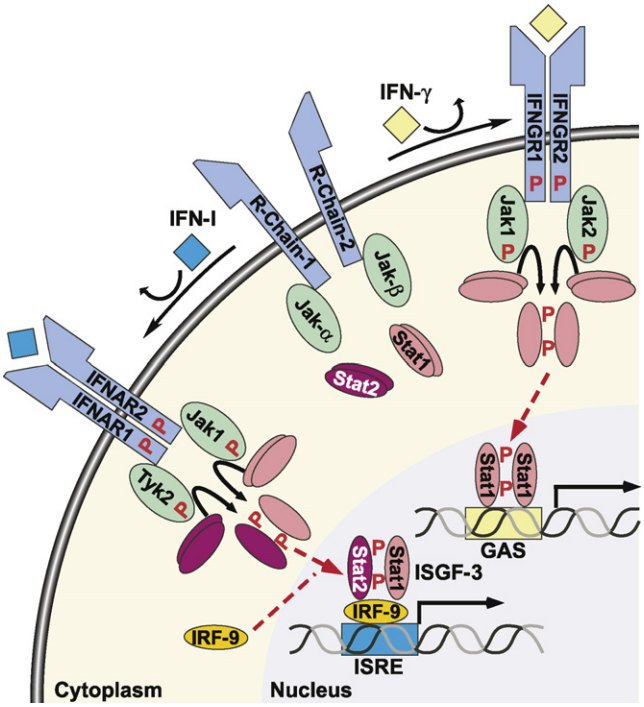


**Fig. 2.** The IFN-I and IFN-γ signaling paradigm. Upon binding to its dimeric receptor (IFNAR1 and IFNAR2), *type I IFN* promotes the apposition of two receptor-associated JAKs (Jak1 and Tyk2), directing transphosphorylation and activation. The activated JAKs then phosphorylate receptor tyrosine(s), promoting a SH2 domain-dependent recruitment of Stat1 and Stat2. At the receptor, Stat1 and Stat2 are activated by phosphorylation, they heterodimerizes, translocate into the nucleus, and associate with IRF-9 to from ISGF-3, which binds to ISREs to drive the expression of corresponding target genes. Only type I and type III IFNs signal through this pathway. *IFN-γ* directs the activation of a unique dimeric receptor (i.e., IFNGR1 and IFNGR2) by promoting the activation of two receptor-associated JAKs (i.e., Jak1 and Jak2). These JAKs phosphorylate a single IFNGR1 tyrosine, which directs the SH2 domain-dependent recruitment and activation of Stat1. Activated Stat1 homodimers translocate to the nucleus, bind to the members of the GAS family of enhancers and drive the expression of target genes. All four-helix bundle cytokines family, including type I and III IFNs transduce signals through this pathway.

Jak3 expression is limited to lymphoid tissues. Biochemical and genetic studies have genetically and physically linked Jak3 to the common gamma chain (γC), which is associated with members of the lymphoid predominant IL-2 family of cytokine receptors (e.g., receptors for IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21; see Table 1). Consistent with this, Jak3 and γC knockout mice both exhibit Severe Combined Immunodeficiency (SCID)-like defects, highlighting Jak3 as an appealing therapeutic target [51–54].

As outlined above, Tyk2 was initially associated with IFN-I response. However, subsequent biochemical and genetic studies implicated this kinase in the response to IL-12 and IL-23, as well as several members of the IL-6 and IL-10 receptor families. Intriguingly, loss of Tyk2 has been associated with distinct phenotypes in humans and mice. Whereas Tyk2 knockout mice feature modest cytokine defects and a proclivity towards type 2 (i.e., allergic) T-cell responses [55–57], Tyk2 deficient humans exhibit a severe allergic phenotype that has been attributed to an impaired antimicrobial response [58]. In mice, Tyk2 may play a more important role in integrating the response to multiple cytokines [59].

## 4. The STAT family of transcription factors

The seven mammals STAT (Stats1–6, 5a and 5b) range in size from 750 and 900 amino acids (see Fig. 1). Both their chromosomal distribution and homologues in model eukaryotes, suggest this family arose from a single primordial gene, as the need for cell-to-cell communication increased [60,61]. Stat3 and Stat5 are most closely related to those homologues found in model eukaryotes, like *Dictyostelium*, *C. elegans* and *Drosophila* (see article number 4; [60]). Notably, the single *Drosophila* STAT transduces signals through a "classic" JAK–STAT pathway, whereas homologues in *Dictyostelium* and *C. elegans* appear to signal through alternative pathway(s).

STATs can be divided into 7 structurally and/or functionally conserved domains (see Fig. 1; [24,25,62,63]). (1) The *Amino Terminal Domain* ($NH_2$; ~125 amino acids) is well conserved and promotes the formation of homotypic dimers among unphosphorylated STATs [63–66]. This not only assures that STATs remain in an "off" conformation in the resting state, but also facilitates delivery and subsequent activation of STAT pairs at the receptor.

(2) The *Coiled-Coil Domain* (amino acids ~135 to ~315) consists of a potentially dynamic four-helix bundle that protrudes laterally (~80 Å) from the core. This domain associates with regulatory proteins and has also been implicated in controlling the process of nuclear import and export (see article number 3; [46,67]). (3) The *DNA Binding Domain* (DBD; amino acids ~320 to ~480) is also well conserved and mediates a robust binding to GAS palindromes. All activated STAT homodimers, except Stat2, directly bind GAS elements. The DBD has also been implicated in the regulation of nuclear import and export (see also article number 3; [24,25,67,68]). (4) The *Linker Domain* (amino acids ~480 to ~575) structurally translates active dimerization to the DNA binding motif. Studies also suggest that it regulates a process of continual basal (i.e., in resting cells) nuclear export [69]. (5) The *SH2 Domain* (amino acids ~575 to ~680) is the most highly conserved motif. It mediates specific recruitment to receptor chains, as well as the formation of active STAT dimers [26,27,46]. It has been argued that this domain may represent the primordial SH2 domain [25]. (6) The *Tyrosine Activation Motif* consists of a conserved tyrosine along with 5–7 specific carboxy terminal amino acids, usually near residue 700. Like the corresponding SH2 domain, this motif resides on the exposed surface of the inactive homodimer, facilitating its JAK-dependent phosphorylation during receptor recruitment [63,64]. Upon phosphorylation, this motif is recognized and bound by the corresponding SH2 domain of the partner STAT, directing the critical structural changes required for an active conformation [62–64]. (7) The *Transcriptional Activation Domain* (TAD) resides at the carboxy terminus and is highly variable in length and sequence between STAT family members. However, for each specific STAT, except Stat2, this sequence is conserved in humans and mice [46,70]. Many TADs include conserved serine phosphorylation sites that facilitate the recruitment of coactivators (e.g., CBP, p300 and the MCM complex; see below [71,72]). STAT TADs also recruit pol II, HATs (e.g., PCAF, GCN5 and NcoA-1 [73–75]), chromatin modifying complexes (e.g., BAF and SWI2-SNF2 [76–79]) and HDACs [80,81]. The STAT TAD also appears to regulate protein stability. Specifically, Stat4, Stat5 and Stat6 can be targeted for ubiquitin-dependent destruction, whereas Stat1, Stat2 and Stat3 are more stable [82,83]. Finally, a number of native carboxy terminally truncated STAT isoforms have been shown to direct unique programs of gene expression through their association with other transcription factors (e.g., Stat1β with Stat2 in ISGF-3 and Stat3β with c-jun [5,84,85]).

### 4.1. Stat1

Consistent with biochemical studies linking Stat1 activation with the biological response to IFN-α (i.e., ISGF-3 [7]) and IFN-γ (i.e., a Stat1 homodimer; see Fig. 2; [9]), Stat1 knockout mice exhibit profound defects in their biological response to type I and type II IFNs [86,87]. However, defects in the biological response to other Stat1 activating ligands (e.g., IL-6 and EGF) are considerably more modest. In humans, defects in IFN-γ—Stat1 axis have been intimately linked with increased susceptibility to mycobacterial infection [88,89]. In addition to their role in directing an effective innate response to intracellular bacteria, Stat1 target genes have been associated with suppression of cellular proliferation [46]. This contrasts the pro-proliferative and anti-inflammatory activities linked to Stat3 (see below; see also article numbers 4, 11 and 12), and raises the possibility that Stat1 and Stat3 serve to functionally antagonize each other (e.g., [90]). Finally, Stat1β, which arises as an alternate splice isoform, is missing the 39 amino acid carboxy terminal TAD [7]. Although Stat1β appears to be fully functional in the response to IFN-Is, it is defective in the response to IFN-γ, where it may actually antagonize Stat1α (i.e., full length) activity [91].

### 4.2. Stat2

Most divergent in sequence and function, Stat2 neither appears to homodimerize nor bind DNA directly. Rather, in an exclusive response to type I (and type III [44,45]) IFNs, Stat2 forms an active heterodimer with either Stat1α or Stat1β. This Stat1-Stat2 dimer associates with a unique DNA binding protein, IRF-9, to drive a Stat2-TAD-dependent expression of target genes [5,92,93]. This appears to include the expression of microRNAs [94]. Curiously, the Stat2 TAD is not conserved between humans and mice [70]. Consistent with these biochemical studies, Stat2 knockout mice exhibit profound defects in their biological response to type I IFNs and likely type III IFNs [95]. More detailed analysis of IFN-I response in Stat2[−/−] tissues has revealed a loss in IFN-I autocrine activity, abnormal DC maturation and a loss in SOCS-1 expression [95–97]. This latter response appears to assure that the response to IFN-Is is considerably more transient than the response to IFN-γ [97,154].

### 4.3. Stat3

Initially identified as an IL-6-dependent transcription factor [98], two alternate and functionally distinct splice isoforms have been rigorously characterized, Stat3α (full length) and Stat3β (missing the carboxy terminal TAD [84,99]). Reflecting Stat3's ancient lineage [61], biochemical and genetic studies have underscored the important role this transcription factor plays in transducing signals for the IL-6 family (IL-6, IL-11, IL-31, LIF, CNTF, CLC/CLF, NP, CT1, OSM), the IL-10 family (IL-10, IL-19, IL-20, IL-22, IL-24, IL-26), as well as G-CSF, Leptin, IL-21, IL-27 and potentially IFN-Is (see Table 1; see also article numbers 4 and 12; [47,60,100]). Consistent with a broad range in activity, Stat3 knockout mice exhibit an early embryonic lethal phenotype (at E6.5–7.5 [101]). Tissue specific Stat3 knockouts have been associated with an increased inflammatory response, altered energy homeostasis, developmental defects and a decreased oncogenic potential [102–111]. The inflammatory phenotype associated with Stat3 deficiency likely reflects its role in directing the response to anti-inflammatory cytokines (e.g., IL-10 family and IL-27), as well as Th17 and regulatory T-cells activity (see article number 10; [100,112–114]). In contrast, Stat3 hyper-activation has been associated with immune suppression and transformation [115,116]. Thus, Stat3's role in transformation is likely to be complex [110,117,156].

### 4.4. Stat4

Identified in a search for Stat1 homologues [36,37], Stat4 was mapped adjacent to Stat1 on murine chromosome 2 [118]. Subsequently, Stat4 was found to transduce signals for IL-12 (consisting of p40 + p35 subunits) and more recently IL-23 (consisting of p19 + p35 subunits [119,120]). Specifically, Stat4 directs the IL-12-dependent polarization of naïve CD4+ T-cells towards IFN-γ secreting Th1 cells, as well as the activation of IFN-γ secreting NK cells [121–123]. Stat4 also plays an important role in the IL-23-dependent polarization of naïve CD4+ T-cells into Th17 cells (see article number 10; [100,113]). Finally, studies have highlighted the ability of other cytokines to synergize with IL-12 in stimulating potent Stat4 activation through their capacity to promote Stat4 serine phosphorylation [124].

### 4.5. Stat5

Initially identified as prolactin and IL-3 stimulated transcription factor [40–42], Stat5a and Stat5b arise from a set of tandemly duplicated genes adjacent to Stat3 on murine chromosome 17 [61,118]. Like Stat3, these two STATs exhibit the highest degree of

homology with invertebrate STATs and are functionally pleiotropic (see article number 4; [46,47,61]). Biochemical and genetic studies have revealed that Stat5a and Stat5b direct the biological response for the IL-3 family (IL-3, IL-5 and GM-CSF), single chain family (e.g., GH, Prl, Tpo and Epo) and $\gamma_C$ family (i.e., the IL-2, IL-7, IL-9, IL-15 and IL-21) of cytokine receptors (see Table 1; [46,47]). At 96% amino acid identity, these two STATs appear to be functionally redundant, excepting the response to Prl, which favors Stat5a, and GH, which favors Stat5b (see article number 6; [40,125–127]). Deletion of the entire Stat5a + Stat5b locus has underscored the critical role Stat5 plays in driving both erythropoiesis and lymphopoiesis [128].

### 4.6. Stat6

Initially identified as IL-4Stat, Stat6 was subsequently shown to transduce signals for IL-13, which shares a receptor chain with IL-4 [38,39,46,47,121]. Reflecting its evolutionary juxta-position with Stat2 on murine chromosome 12 [118], Stat6 is divergent in sequence and features a large (~150 residue), functionally unique TAD [118,129]. Intriguingly, Stat6 homodimers bind to a GAS element that features an additional central nucleotide, providing an opportunity to activate a distinct subset of GAS-driven genes. Stat6 knockout mice have underscored the critical role this STAT plays in directing IL-4/IL-13 dependent: Th2-cell polarization; B-cell function (e.g., proliferation, maturation, MHC-II and IgE expression); and mast cell activity [121,130–132].

## 5. Regulating STAT activity

A characteristic feature of JAK–STAT signaling is its rapid onset and decay. Consistent with this, STATs associate with several classes of regulators, including those that promote covalent modifications in addition to canonical tyrosine phosphorylation. The best-characterized negative regulators include phosphatases, nuclear import/export machinery and members of the SOCS family. However, other negative regulators like PIAS and Nmi have been reported [133,134].

### 5.1. Covalent STAT modifications

STATs appear to undergo covalent modifications in addition to canonical tyrosine phosphorylation, most notably serine phosphorylation (see below). However, there are also several reports of acetylation and *O*-glycosylation [135–138]. Intriguingly, there is biochemical and genetic evidence for the ubiquitin-directed decay of Stat4, Stat5 and Stat6 [82,83]. However, evidence supporting a role for R-methylation (Stat1) and SUMOylation (Stat1) in controlling STAT activity remains more controversial [139–141].

With the potential exception of Stat2, all STATs are phosphorylated on at least one serine residue in their TAD, a modification that promotes transcriptional activity (see above [71,72,124,142,143]). However, it is not clear where this modification occurs. Conserved phosphorylation sites include, a PMS*P motif (S727 in STATs 1 and 3; S721 in Stat4), a PS*P motif (S725 in Stat5a; S730 in Stat5b) and a SS*PD motif (S756 in Stat6 [82]). Stat1 and Stat5 feature at least one additional serine phosphorylation site in their TAD, S708 and S779, respectively. Biochemical studies have implicated a number of kinases in STAT serine phosphorylation, including MAP kinases (e.g., p38: STATs 1, 3 and 4; ERK: Stat3 and Stat5; JNK: Stat3), PKCδ (Stat1 and Stat3), mTOR (Stat3), NLK (Stat3), CaMKII and IKKε (Stat1 [71,144,145]). However, genetic evidence supporting a role for theses kinases in regulating STAT activity is considerably more limited.

### 5.2. Phosphatases

Since kinases play an important role in cytokine response, it is not surprising that phosphatases, including SHP-1, SHP-2 and CD45 have been implicated in returning cytokine receptors and JAKs back to their basal unphosphorylated state ([60,146]; see also article number 12). Likewise, SHP-2, PTP1B, TC-PTP and PTP-BL have been implicated in restoring STATs to basal unphosphorylated state [146–151]. However, only two of these phosphatases, SHP-2 and TC-PTP, appear to exhibit robust activity in the nucleus, where STAT desphosphorylation has been linked to STAT nuclear export [67,152,153].

### 5.3. Nuclear import–export

The dramatic nuclear accumulation of activated STATs belie the complex process regulating STAT localization (see article number 3; [67,69,152]). The predominately cytosolic localization of inactive STATs reflects a steady state, where continuous basal nuclear import is balanced by continuous basal export. Upon stimulation, this balance is shifted towards STAT nuclear accumulation, and then in the opposite direction during the process of signal decay. Of note, multiple nuclear export sequence (NES) and nuclear localization sequence (NLS) elements have been implicated in this dynamic process [67,69,152].

#### 5.3.1. The SOCS family

A subset of the SOCS proteins constitute a classic negative "feedback loop" [14,154]. In the basal state SOCS proteins are expressed at low levels. However, upon stimulation the expression of these STAT target genes is rapidly induced, whereupon they function to antagonize further STAT activation. Gene targeting studies have underscored important roles for SOCS-1, SOCS-2 and SOCS-3 in antagonizing the IFN-γ–Stat1, IL-12–Stat4, IL-4–Stat6, GH–Stat5 and IL-6–Stat3 axes.

## 6. A bright future

Characterization of the ability of IFNs to direct an antiviral response led to the identification of the JAK–STAT signaling cascade, and provided insight into how the more than 50 members of the four-helix bundle cytokine family transduce their biological response (see Table 1). Future studies are likely to exploit conditional gene targeting, as well as improving pharmaceutical agents to explore how these pathways regulate immune homeostasis in vivo. This is not only likely to include the discovery of additional regulators, ligands and receptors, but also provide new insight into "crosstalk" with other signaling cascades.

## References

[1] Isaacs A, Lindenmann J. Virus interference. I. The interferon. Proc Roy Soc Lond B Biol Sci 1957;147:258–67.
[2] Pestka S. The human interferon alpha species and receptors. Biopolymers 2000;55:254–87.
[3] Levy D, Larner A, Chaudhuri A, Babiss LE, Darnell Jr JE. Interferon-stimulated transcription: isolation of an inducible gene and identification of its regulatory region. Proc Natl Acad Sci USA 1986;83:8929–33.
[4] Reich N, Evans B, Levy D, Fahey D, Knight Jr E, Darnell Jr JE. Interferon-induced transcription of a gene encoding a 15-kDa protein depends on an upstream enhancer element. Proc Natl Acad Sci USA 1987;84:6394–8.
[5] Fu X-Y, Schindler C, Improta T, Aebersold R, Darnell JE. The proteins of ISGF-3, the IFN-α induced transcription activator, define a new family of signal transducers. Proc Natl Acad Sci USA 1992;89:7840–3.
[6] Kessler DS, Levy DE, Darnell Jr JE. Two interferon-induced nuclear factors bind a single promoter element in interferon-stimulated genes. Proc Natl Acad Sci USA 1988;85:8521–5.
[7] Schindler C, Fu XY, Improta T, Aebersold R, Darnell Jr JE. Proteins of transcription factor ISGF-3: one gene encodes the 91- and 84-kDa ISGF-3 proteins

that are activated by interferon alpha. Proc Natl Acad Sci USA 1992;89: 7836–9.
[8] Schindler C, Shuai K, Prezioso VR, Darnell Jr JE. Interferon-dependent tyrosine phosphorylation of a latent cytoplasmic transcription factor. Science 1992;257:809–13.
[9] Shuai K, Schindler C, Prezioso V, Darnell JE. Activation of transcription by IFN-γ: tyrosine phosphorylation of a 91-kDa DNA binding protein. Science 1992;258:1808–12.
[10] Velazquez L, Fellous M, Stark GR, Pellegrini S. A protein tyrosine kinase in the interferon alpha/beta signaling pathway. Cell 1992;70:313–22.
[11] Levy DE, Darnell Jr JE. Stats: transcriptional control and biological impact. Nat Rev Mol Cell Biol 2002;3:651–62.
[12] Decker T, Muller M, Stockinger S. The yin and yang of type I interferon activity in bacterial infection. Nat Rev Immunol 2005;5:675–87.
[13] O'Shea JJ, Gadina M, Schreiber RD. Cytokine signaling in 2002: new surprises in the Jak/Stat pathway. Cell 2002;109(Suppl):S121–31.
[14] Alexander WS, Hilton DJ. The role of suppressors of cytokine signaling (SOCS) proteins in regulation of the immune response. Annu Rev Immunol 2004;22:503–29.
[15] Levy D, Reich N, Kessler D, Pine R, Darnell Jr JE. Transcriptional regulation of interferon-stimulated genes: a DNA response element and induced proteins that recognize it. Cold Spring Harb Symp Quant Biol 1988;53(Pt 2):799–802.
[16] Fu XY, Kessler DS, Veals SA, Levy DE, Darnell Jr JE. ISGF3, the transcriptional activator induced by interferon alpha, consists of multiple interacting polypeptide chains. Proc Natl Acad Sci USA 1990;87:8555–9.
[17] Levy DE, Kessler DS, Pine R, Darnell Jr JE. Cytoplasmic activation of ISGF3, the positive regulator of interferon-alpha-stimulated transcription, reconstituted in vitro. Genes Dev 1989;3:1362–71.
[18] Veals SA, Schindler C, Leonard D, Fu XY, Aebersold R, Darnell Jr JE, et al. Subunit of an alpha-interferon-responsive transcription factor is related to interferon regulatory factor and Myb families of DNA-binding proteins. Mol Cell Biol 1992;12:3315–24.
[19] Shuai K, Schindler C, Prezioso VR, Darnell Jr JE. Activation of transcription by IFN-gamma: tyrosine phosphorylation of a 91-kD DNA binding protein. Science 1992;258:1808–12.
[20] Lew DJ, Decker T, Strehlow I, Darnell JE. Overlapping elements in the guanylate-binding protein gene promoter mediate transcriptional induction by alpha and gamma interferons. Mol Cell Biol 1991;11:182–91.
[21] Eilers A, Seegert D, Schindler C, Baccarini M, Decker T. The response of gamma interferon activation factor is under developmental control in cells of the macrophage lineage. Mol Cell Biol 1993;13:3245–54.
[22] Kanno Y, Kozak CA, Schindler C, Driggers PH, Ennist DL, Gleason SL, et al. The genomic structure of the murine ICSBP gene reveals the presence of the gamma interferon-responsive element, to which an ISGF3 alpha subunit (or similar) molecule binds. Mol Cell Biol 1993;13:3951–63.
[23] Pearse RN, Feinman R, Shuai K, Darnell Jr JE, Ravetch JV. Interferon gamma-induced transcription of the high-affinity Fc receptor for IgG requires assembly of a complex that includes the 91-kDa subunit of transcription factor ISGF3. Proc Natl Acad Sci USA 1993;90:4314–8.
[24] Becker S, Groner B, Muller CW. Three-dimensional structure of the Stat3beta homodimer bound to DNA. Nature 1998;394:145–51.
[25] Chen X, Vinkemeier U, Zhao Y, Jeruzalmi D, Darnell Jr JE, Kuriyan J. Crystal structure of a tyrosine phosphorylated STAT-1 dimer bound to DNA. Cell 1998;93:827–39.
[26] Gupta P, Blazar BR, Gupta K, Verfaillie CM. Human CD34(+) bone marrow cells regulate stromal production of interleukin-6 and granulocyte colony-stimulating factor and increase the colony-stimulating activity of stroma. Blood 1998;91:3724–33.
[27] Shuai K, Horvath CM, Huang LH, Qureshi SA, Cowburn D, Darnell Jr JE. Interferon activation of the transcription factor Stat91 involves dimerization through SH2-phosphotyrosyl peptide interactions. Cell 1994;76:821–8.
[28] Firmbach-Kraft I, Byers M, Shows T, Dalla-Favera R, Krolewski JJ. tyk2, prototype of a novel class of non-receptor tyrosine kinase genes. Oncogene 1990;5:1329–36.
[29] Wilks AF, Harpur AG, Kurban RR, Ralph SJ, Zurcher G, Ziemiecki A. Two novel protein-tyrosine kinases, each with a second phosphotransferase-related catalytic domain, define a new class of protein kinase. Mol Cell Biol 1991;11:2057–65.
[30] Silvennoinen O, Ihle JN, Schlessinger J, Levy DE. Interferon-induced nuclear signalling by Jak protein tyrosine kinases. Nature 1993;366:583–5.
[31] Watling D, Guschin D, Muller M, Silvennoinen O, Witthuhn BA, Quelle FW, et al. Complementation by the protein tyrosine kinase JAK2 of a mutant cell line defective in the interferon-gamma signal transduction pathway. Nature 1993;366:166–70.
[32] Argetsinger LS, Campbell GS, Yang X, Witthuhn BA, Silvennoinen O, Ihle JN, et al. Identification of JAK2 as a growth hormone receptor-associated tyrosine kinase. Cell 1993;74:237–44.
[33] Müller M, Briscoe J, Laxton C, Guschin D, Ziemiecki A, Silvennoinen O, et al. The protein tyrosine kinase JAK1 complements defects in interferon- alpha/beta and -gamma signal transduction. Nature 1993;366:129–35.
[34] Bonni A, Frank DA, Schindler C, Greenberg ME. Characterization of a pathway for ciliary neurotrophic factor signaling to the nucleus. Science 1993;262:1575–9.
[35] Akira S, Nishio Y, Inoue M, Wang XJ, Wei S, Matsusaka T, et al. Molecular cloning of APRF, a novel IFN-stimulated gene factor 3 p91-related transcription factor involved in the gp130-mediated signaling pathway. Cell 1994;77:63–71.
[36] Yamamoto K, Quelle FW, Thierfelder WE, Kreider BL, Gilbert DJ, Jenkins NA, et al. Stat4, a novel gamma interferon activation site-binding protein expressed in early myeloid differentiation. Mol Cell Biol 1994;14:4342–9.
[37] Zhong Z, Wen Z, Darnell Jr JE. Stat3 and Stat4: members of the family of signal transducers and activators of transcription. Proc Natl Acad Sci USA 1994;91:4806–10.
[38] Schindler C, Kashleva H, Pernis A, Pine R, Rothman P. STF-IL4: a novel IL-4 induced signal transducing factor. EMBO J 1994;13:1350–6.
[39] Hou J, Schindler U, Henzel WJ, Ho TC, Brasseur M, McKnight SL. An interleukin-4-induced transcription factor: IL-4 Stat. Science 1994;265:1701–6.
[40] Azam M, Erdjument-Bromage H, Kreider BL, Xia M, Quelle F, Basu R, et al. Interleukin-3 signals through multiple isoforms of Stat5. EMBO J 1995;14:1402–11.
[41] Mui AL, Wakao H, O'Farrell AM, Harada N, Miyajima A. Interleukin-3, granulocyte-macrophage colony stimulating factor and interleukin-5 transduce signals through two STAT5 homologs. EMBO J 1995;14:1166–75.
[42] Wakao H, Gouilleux F, Groner B. Mammary gland factor (MGF) is a novel member of the cytokine regulated transcription factor gene family and confers the prolactin response. EMBO J 1994;13:2182–91.
[43] Kawamura M, McVicar DW, Johnston JA, Blake TB, Chen YQ, Lal BK, et al. Molecular cloning of L-JAK, a Janus family protein-tyrosine kinase expressed in natural killer cells and activated leukocytes. Proc Natl Acad Sci USA 1994;91:6374–8.
[44] Kotenko SV, Gallagher G, Baurin VV, Lewis-Antes A, Shen M, Shah NK, et al. IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex. Nat Immunol 2003;4:69–77.
[45] Sheppard P, Kindsvogel W, Xu W, Henderson K, Schlutsmeyer S, Whitmore TE, et al. IL-28, IL-29 and their class II cytokine receptor IL-28R. Nat Immunol 2003;4:63–8.
[46] Schindler C, Levy DE, Decker T. JAK–STAT signaling: from interferons to cytokines. J Biol Chem 2007;282:20059–63.
[47] Murray PJ. The JAK–STAT signaling pathway: input and output integration. J Immunol 2007;178:2623–9.
[48] Rodig SJ, Meraz MA, White JM, Lampe PA, Riley JK, Arthur CD, et al. Disruption of the Jak1 gene demonstrates obligatory and nonredundant roles of the Jaks in cytokine-induced biologic responses. Cell 1998;93:373–83.
[49] Neubauer H, Cumano A, Mueller M, Wu H, Huffstadt U, Pfeffer K. Jak2 deficiency defines an essential developmental checkpoint in definitive hematopoiesis. Cell 1998;93:397–409.
[50] Parganas E, Wang D, Stravopodis D, Topham DJ, Marine JC, Teglund S, et al. Jak2 is essential for signaling through a variety of cytokine receptors. Cell 1998;93:385–95.
[51] Tefferi A. JAK and MPL mutations in myeloid malignancies. Leuk Lymphoma 2008;49:388–97.
[52] Nosaka T, vanDeursen JM, Tripp RA, Thierfelder WE, Witthuhn BA, McMickle AP, et al. Defective lymphoid development in mice lacking Jak3. Science 1995;270:800–2.
[53] Park SY, Saijo K, Takahashi T, Osawa M, Arase H, Hirayama N, et al. Developmental defects of lymphoid cells in Jak3 kinase-deficient mice. Immunity 1995;3:771–82.
[54] Thomis DC, Gurniak CB, Tivol E, Sharpe AH, Berg LJ. Defects in B lymphocyte maturation and T lymphocyte activation in mice lacking Jak3. Science 1995;270:794–7.
[55] Seto Y, Nakajima H, Suto A, Shimoda K, Saito Y, Nakayama KI, et al. Enhanced Th2 cell-mediated allergic inflammation in Tyk2-deficient mice. J Immunol 2003;170:1077–83.
[56] Karaghiosoff M, Neubauer H, Lassnig C, Kovarik P, Schindler H, Pircher H, et al. Partial impairment of cytokine responses in Tyk2-deficient mice. Immunity 2000;13:549–60.
[57] Shimoda K, Kato K, Aoki K, Matsuda T, Miyamoto A, Shibamori M, et al. Tyk2 plays a restricted role in IFN alpha signaling, although it is required for IL-12-mediated T cell function. Immunity 2000;13:561–71.
[58] Minegishi Y, Saito M, Morio T, Watanabe K, Agematsu K, Tsuchiya S, et al. Human tyrosine kinase 2 deficiency reveals its requisite roles in multiple cytokine signals involved in innate and acquired immunity. Immunity 2006;25:745–55.
[59] Karaghiosoff M, Steinborn R, Kovarik P, Kriegshauser G, Baccarini M, Donabauer B, et al. Central role for type I interferons and Tyk2 in lipopolysaccharide-induced endotoxin shock. Nat Immunol 2003;4:471–7.
[60] Kisseleva T, Bhattacharya S, Braunstein J, Schindler CW. Signaling through the JAK/STAT pathway, recent advances and future challenges. Gene 2002;285:1–24.
[61] Miyoshi K, Cui Y, Riedlinger G, Lehoczky J, Zon L, Oka T, et al. Structure of the mouse stat 3/5 locus: evolution from drosophila to zebrafish to mouse. Genomics 2001;71:150–5.
[62] Neculai D, Neculai AM, Verrier S, Straub K, Klumpp K, Pfitzner E, et al. Structure of the unphosphorylated STAT5a dimer. J Biol Chem 2005;280:40782–7.
[63] Mao X, Ren Z, Parker GN, Sondermann H, Pastorello MA, Wang W, et al. Structural bases of unphosphorylated STAT1 association and receptor binding. Mol Cell 2005;17:761–71.
[64] Mertens C, Zhong M, Krishnaraj R, Zou W, Chen X, Darnell Jr JE. Dephosphorylation of phosphotyrosine on STAT1 dimers requires extensive spatial

reorientation of the monomers facilitated by the N-terminal domain. Genes Dev 2006;20:3372–81.
[65] Zhong M, Henriksen MA, Takeuchi K, Schaefer O, Liu B, ten Hoeve J, et al. Implications of an antiparallel dimeric structure of nonphosphorylated STAT1 for the activation-inactivation cycle. Proc Natl Acad Sci USA 2005;102:3966–71.
[66] Braunstein J, Brutsaert S, Olson R, Schindler C. STATs dimerize in the absence of phosphorylation. J Biol Chem 2003;278:34133–40.
[67] McBride KM, Reich NC. The ins and outs of STAT1 nuclear transport. Sci STKE 2003;2003:RE13.
[68] Meyer T, Marg A, Lemke P, Wiesner B, Vinkemeier U. DNA binding controls inactivation and nuclear accumulation of the transcription factor Stat1. Genes Dev 2003;17:1992–2005.
[69] Bhattacharya S, Schindler C. Regulation of Stat3 nuclear export. J Clin Invest 2003;111:553–9.
[70] Park C, Lecomte MJ, Schindler C. Murine Stat2 is uncharacteristically divergent. Nucleic Acids Res 1999;27:4191–9.
[71] Decker T, Müller M, Kovarik P. Regulation of Stats by posttranslational modification. In: Seghal PB, Hirano T, Levy DE, editors. Signal transducers and activators of transcription (Stats): activation and biology. Kluwer Academic; 2003.
[72] Ramsauer K, Farlik M, Zupkovitz G, Seiser C, Kroger A, Hauser H, et al. Distinct modes of action applied by transcription factors STAT1 and IRF1 to initiate transcription of the IFN-{gamma}-inducible gbp2 gene. Proc Natl Acad Sci USA 2007;104:2849–54.
[73] Litterst CM, Pfitzner E. An LXXLL motif in the transactivation domain of STAT6 mediates recruitment of NCoA-1/SRC-1. J Biol Chem 2002;277:36052–60.
[74] Giraud S, Bienvenu F, Avril S, Gascan H, Heery DM, Coqueret O. Functional interaction of STAT3 transcription factor with the coactivator NcoA/SRC1a. J Biol Chem 2002;277:8004–11.
[75] Paulson M, Press C, Smith E, Tanese N, Levy DE. IFN-Stimulated transcription through a TBP-free acetyltransferase complex escapes viral shutoff. Nat Cell Biol 2002;4:140–7.
[76] Letimier FA, Passini N, Gasparian S, Bianchi E, Rogge L. Chromatin remodeling by the SWI/SNF-like BAF complex and STAT4 activation synergistically induce IL-12Rbeta2 expression during human Th1 cell differentiation. EMBO J 2007;26:1292–302.
[77] Pattenden SG, Klose R, Karaskov E, Bremner R. Interferon-gamma-induced chromatin remodeling at the CIITA locus is BRG1 dependent. EMBO J 2002;21:1978–86.
[78] Liu H, Kang H, Liu R, Chen X, Zhao K. Maximal induction of a subset of interferon target genes requires the chromatin-remodeling activity of the BAF complex. Mol Cell Biol 2002;22:6471–9.
[79] Huang M, Qian F, Hu Y, Ang C, Li Z, Wen Z. Chromatin-remodelling factor BRG1 selectively activates a subset of interferon-alpha-inducible genes. Nat Cell Biol 2002;4:774–81.
[80] Nusinzon I, Horvath CM. Unexpected roles for deacetylation in interferon- and cytokine-induced transcription. J Interferon Cytokine Res 2005;25:745–8.
[81] Rascle A, Johnston JA, Amati B. Deacetylase activity is required for recruitment of the basal transcription machinery and transactivation by STAT5. Mol Cell Biol 2003;23:4162–73.
[82] Wang D, Moriggl R, Stravopodis D, Carpino N, Marine JC, Teglund S, et al. A small amphipathic alpha-helical region is required for transcriptional activities and proteasome-dependent turnover of the tyrosine-phosphorylated Stat5. EMBO J 2000;19:392–9.
[83] Tanaka T, Soriano MA, Grusby MJ. SLIM is a nuclear ubiquitin E3 ligase that negatively regulates STAT signaling. Immunity 2005;22:729–36.
[84] Maritano D, Sugrue ML, Tininini S, Dewilde S, Strobl B, Fu X, et al. The STAT3 isoforms alpha and beta have unique and specific functions. Nat Immunol 2004;5:401–9.
[85] Ivanov VN, Bhoumik A, Krasilnikov M, Raz R, Owen-Schaub LB, Levy D, et al. Cooperation between STAT3 and c-jun suppresses Fas transcription. Mol Cell 2001;7:517–28.
[86] Durbin JE, Hackenmiller R, Simon MC, Levy DE. Targeted disruption of the mouse Stat1 gene results in compromised innate immunity to viral disease. Cell 1996;84:443–50.
[87] Meraz MA, White JM, Sheehan KC, Bach EA, Rodig SJ, Dighe AS, et al. Targeted disruption of the Stat1 gene in mice reveals unexpected physiologic specificity in the JAK–STAT signaling pathway. Cell 1996;84:431–42.
[88] Chapgier A, Boisson-Dupuis S, Jouanguy E, Vogt G, Feinberg J, Prochnicka-Chalufour A, et al. Novel STAT1 alleles in otherwise healthy patients with mycobacterial disease. PLoS Genet 2006;2:e131.
[89] Jouanguy E, Lamhamedi-Cherradi S, Lammas D, Dorman SE, Fondaneche MC, Dupuis S, et al. A human IFNGR1 small deletion hotspot associated with dominant susceptibility to mycobacterial infection. Nat Genet 1999;21:370–8.
[90] Costa-Pereira AP, Tininini S, Strobl B, Alonzi T, Schlaak JF, Is'harc H, et al. Mutational switch of an IL-6 response to an interferon-gamma -like response. Proc Natl Acad Sci USA 2002;99:8043–7.
[91] Muller M, Laxton C, Briscoe J, Schindler C, Improta T, Darnell Jr JE, et al. Complementation of a mutant cell line: central role of the 91 kDa polypeptide of ISGF3 in the interferon-alpha and -gamma signal transduction pathways. EMBO J 1993;12:4221–8.
[92] Improta T, Schindler C, Horvath CM, Kerr IM, Stark GR, Darnell Jr JE. Transcription factor ISGF-3 formation requires phosphorylated Stat91 protein, but Stat113 protein is phosphorylated independently of Stat91 protein. Proc Natl Acad Sci USA 1994;91:4776–80.
[93] Qureshi SA, Leung S, Kerr IM, Stark GR, Darnell Jr JE. Function of Stat2 protein in transcriptional activation by alpha interferon. Mol Cell Biol 1996;16:288–93.
[94] Pedersen IM, Cheng G, Wieland S, Volinia S, Croce CM, Chisari FV, et al. Interferon modulation of cellular microRNAs as an antiviral mechanism. Nature 2007;449:919–22.
[95] Park C, Li S, Cha E, Schindler C. Immune response in Stat2 knockout mice. Immunity 2000;13:795–804.
[96] Hahm B, Trifilo MJ, Zuniga EI, Oldstone MB. Viruses evade the immune system through type I interferon-mediated STAT2-dependent, but STAT1-independent, signaling. Immunity 2005;22:247–57.
[97] Zhao W, Cha EN, Lee C, Park CY, Schindler C. Stat2-dependent regulation of MHC class II expression. J Immunol 2007;179:463–71.
[98] Akira S, Nishio Y, Inoue M, Wang X-J, Wei S, Matsusaka T, et al. Molecular cloning of APRF, a novel IFN-stimulated gene factor 3 p91-related transcription factor involved in the gp130-mediated signaling pathway. Cell 1994;77:63–71.
[99] Zhang X, Wrzeszczynska MH, Horvath CM, Darnell Jr JE. Interacting regions in Stat3 and c-Jun that participate in cooperative transcriptional activation. Mol Cell Biol 1999;19:7138–46.
[100] Jankovic D, Trinchieri G. IL-10 or not IL-10: that is the question. Nat Immunol 2007;8:1281–3.
[101] Takeda K, Noguchi K, Shi W, Tanaka T, Matsumoto M, Yoshida N, et al. Targeted disruption of the mouse Stat3 gene leads to early embryonic lethality. Proc Natl Acad Sci USA 1997;94:3801–4.
[102] Yoshimatsu T, Kawaguchi D, Oishi K, Takeda K, Akira S, Masuyama N, et al. Non-cell-autonomous action of STAT3 in maintenance of neural precursor cells in the mouse neocortex. Development 2006;133:2553–63.
[103] Hokuto I, Ikegami M, Yoshida M, Takeda K, Akira S, Perl AK, et al. Stat-3 is required for pulmonary homeostasis during hyperoxia. J Clin Invest 2004;113:28–37.
[104] Gao Q, Wolfgang MJ, Neschen S, Morino K, Horvath TL, Shulman GI, et al. Disruption of neural signal transducer and activator of transcription 3 causes obesity, diabetes, infertility, and thermal dysregulation. Proc Natl Acad Sci USA 2004;101:4661–6.
[105] Alonzi T, Maritano D, Gorgoni B, Rizzuto G, Libert C, Poli V. Essential role of STAT3 in the control of the acute-phase response as revealed by inducible gene inactivation [correction of activation] in the liver. Mol Cell Biol 2001;21:1621–32.
[106] Levy DE, Lee CK. What does Stat3 do? J Clin Invest 2002;109:1143–8.
[107] Lee CK, Raz R, Gimeno R, Gertner R, Wistinghausen B, Takeshita K, et al. STAT3 is a negative regulator of granulopoiesis but is not required for G-CSF-dependent differentiation. Immunity 2002;17:63–72.
[108] Chapman RS, Lourenco PC, Tonner E, Flint DJ, Selbert S, Takeda K, et al. Suppression of epithelial apoptosis and delayed mammary gland involution in mice with a conditional knockout of Stat3. Genes Dev 1999;13:2604–16.
[109] Levy DE, Loomis CA. STAT3 signaling and the hyper-IgE syndrome. N Engl J Med 2007;357:1655–8.
[110] de la Iglesia N, Konopka G, Puram SV, Chan JA, Bachoo RM, You MJ, et al. Identification of a PTEN-regulated STAT3 brain tumor suppressor pathway. Genes Dev 2008.
[111] Inghirami G, Chiarle R, Simmons WJ, Piva R, Schlessinger K, Levy DE. New and old functions of STAT3: a pivotal target for individualized treatment of cancer. Cell Cycle 2005;4:1131–3.
[112] Chen Z, Laurence A, O'Shea JJ. Signal transduction pathways and transcriptional regulation in the control of Th17 differentiation. Semin Immunol 2007;19:400–8.
[113] Mathur AN, Chang HC, Zisoulis DG, Stritesky GL, Yu Q, O'Malley JT, et al. Stat3 and Stat4 direct development of IL-17-secreting Th cells. J Immunol 2007;178:4901–7.
[114] Owaki T, Asakawa M, Morishima N, Mizoguchi I, Fukai F, Takeda K, et al. STAT3 is indispensable to IL-27-mediated cell proliferation but not to IL-27-induced Th1 differentiation and suppression of proinflammatory cytokine production. J Immunol 2008;180:2903–11.
[115] Yu H, Kortylewski M, Pardoll D. Crosstalk between cancer and immune cells: role of STAT3 in the tumour microenvironment. Nat Rev Immunol 2007;7:41–51.
[116] Bromberg JF, Wrzeszczynska MH, Devgan G, Zhao Y, Pestell RGC, Albanese Darnell JE. Stat3 as an oncogene. Cell 1999;98:295–303.
[117] Naugler WE, Karin M. The wolf in sheep's clothing: the role of interleukin-6 in immunity, inflammation and cancer. Trends Mol Med 2008;14:109–19.
[118] Copeland NG, Gilbert DJ, Schindler C, Zhong Z, Wen Z, Darnell Jr JE, et al. Distribution of the mammalian Stat gene family in mouse chromosomes. Genomics 1995;29:225–8.
[119] Hunter CA. New IL-12-family members: IL-23 and IL-27, cytokines with divergent functions. Nat Rev Immunol 2005;5:521–31.
[120] Zhong Z, Wen Z, Darnell Jr JE. Stat3: a STAT family member activated by tyrosine phosphorylation in response to epidermal growth factor and interleukin-6. Science 1994;264:95–8.
[121] Wurster AL, Tanaka T, Grusby MJ. The biology of Stat4 and Stat6. Oncogene 2000;19:2577–84.
[122] Thierfelder WE, van Deursen JM, Yamamoto K, Tripp RA, Sarawar SR, Carson RT, et al. Requirement for Stat4 in interleukin-12-mediated responses of natural killer and T cells. Nature 1996;382:171–4.

[123] Kaplan MH, Sun YL, Hoey T, Grusby MJ. Impaired IL-12 responses and enhanced development of Th2 cells in Stat4-deficient mice. Nature 1996;382:174–7.
[124] Morinobu A, Gadina M, Strober W, Visconti R, Fornace A, Montagna C, et al. STAT4 serine phosphorylation is critical for IL-12-induced IFN-gamma production but not for cell proliferation. Proc Natl Acad Sci USA 2002;99:12281–6.
[125] Teglund S, McKay C, Schuetz E, van Deursen JM, Stravopodis D, Wang D, et al. Stat5a and Stat5b proteins have essential and nonessential, or redundant, roles in cytokine responses. Cell 1998;93:841–50.
[126] Udy GB, Towers RP, Snell RG, Wilkins RJ, Park SH, Ram PA, et al. Requirement of Stat5b for sexual dimorphism of body growth rates and liver gene expression. Proc Natl Acad Sci USA 1997;94:7239–44.
[127] Cui Y, Riedlinger G, Miyoshi K, Tang W, Li C, Deng CX, et al. Inactivation of Stat5 in mouse mammary epithelium during pregnancy reveals distinct functions in cell proliferation, survival, and differentiation. Mol Cell Biol 2004;24:8037–47.
[128] Yao Z, Cui Y, Watford WT, Bream JH, Yamaoka K, Hissong BD, et al. Stat5a/b are essential for normal lymphoid development and differentiation. Proc Natl Acad Sci USA 2006;103:1000–5.
[129] Hebenstreit D, Wirnsberger G, Horejs-Hoeck J, Duschl A. Signaling mechanisms, interaction partners, and target genes of STAT6. Cytokine Growth Factor Rev 2006;17:173–88.
[130] Takeda K, Tanaka T, Shi W, Matsumoto M, Minami M, Kashiwamura S, et al. Essential role of Stat6 in IL-4 signalling. Nature 1996;380:627–30.
[131] Shimoda K, van Deursen J, Sangster MY, Sarawar SR, Carson RT, Tripp RA, et al. Lack of IL-4-induced Th2 response and IgE class switching in mice with disrupted Stat6 gene. Nature 1996;380:630–3.
[132] Kaplan MH, Schindler U, Smiley ST, Grusby MJ. Stat6 is required for mediating responses to IL-4 and for development of Th2 cells. Immunity 1996;4:313–9.
[133] Shuai K. Modulation of STAT signaling by STAT-interacting proteins. Oncogene 2000;19:2638–44.
[134] Zhu M, John S, Berg M, Leonard WJ. Functional association of Nmi with Stat5 and Stat1 in IL-2- and IFNgamma-mediated signaling. Cell 1999;96:121–30.
[135] Kramer OH, Baus D, Knauer SK, Stein S, Jager E, Stauber RH, et al. Acetylation of Stat1 modulates NF-kappaB activity. Genes Dev 2006;20:473–85.
[136] Nadiminty N, Lou W, Lee SO, Lin X, Trump DL, Gao AC. Stat3 activation of NF-{kappa}B p100 processing involves CBP/p300-mediated acetylation. Proc Natl Acad Sci USA 2006;103:7264–9.
[137] Yuan ZL, Guan YJ, Chatterjee D, Chin YE. Stat3 dimerization regulated by reversible acetylation of a single lysine residue. Science 2005;307:269–73.
[138] Gewinner C, Hart G, Zachara N, Cole R, Beisenherz-Huss C, Groner B. The coactivator of transcription CREB-binding protein interacts preferentially with the glycosylated form of Stat5. J Biol Chem 2004;279:3563–72.
[139] Song L, Bhattacharya S, Yunus AA, Lima CD, Schindler C. Stat1 and SUMO modification. Blood 2006;108:3237–44.
[140] Komyod W, Bauer UM, Heinrich PC, Haan S, Behrmann I. Are STATS arginine-methylated? J Biol Chem 2005;280:21700–5.
[141] Meissner T, Krause E, Lodige I, Vinkemeier U. Arginine methylation of STAT1: a reassessment. Cell 2004, 119; 587–9; discussion 589–90.
[142] Varinou L, Ramsauer K, Karaghiosoff M, Kolbe T, Pfeffer K, Muller M, et al. Phosphorylation of the Stat1 transactivation domain is required for full-fledged IFN-gamma-dependent innate immunity. Immunity 2003;19:793–802.
[143] Shen Y, Schlessinger K, Zhu X, Meffre E, Quimby F, Levy DE, et al. Essential role of STAT3 in postnatal survival and growth revealed by mice lacking STAT3 serine 727 phosphorylation. Mol Cell Biol 2004;24:407–19.
[144] Kojima H, Sasaki T, Ishitani T, Iemura S, Zhao H, Kaneko S, et al. STAT3 regulates Nemo-like kinase by mediating its interaction with IL-6-stimulated TGFbeta-activated kinase 1 for STAT3 Ser-727 phosphorylation. Proc Natl Acad Sci USA 2005;102:4524–9.
[145] Tenoever BR, Ng SL, Chua MA, McWhirter SM, Garcia-Sastre A, Maniatis T. Multiple functions of the IKK-related kinase IKKepsilon in interferon-mediated antiviral immunity. Science 2007;315:1274–8.
[146] Mustelin T, Vang T, Bottini N. Protein tyrosine phosphatases and the immune response. Nat Rev Immunol 2005;5:43–57.
[147] Nakahira M, Tanaka T, Robson BE, Mizgerd JP, Grusby MJ. Regulation of signal transducer and activator of transcription signaling by the tyrosine phosphatase PTP-BL. Immunity 2007;26:163–76.
[148] Irie-Sasaki J, Sasaki T, Matsumoto W, Opavsky A, Cheng M, Welstead G, et al. CD45 is a JAK phosphatase and negatively regulates cytokine receptor signalling. Nature 2001;409:349–54.
[149] Haque SJ, Harbor P, Tabrizi M, Yi T, Williams BR. Protein-tyrosine phosphatase Shp-1 is a negative regulator of IL-4- and IL-13-dependent signal transduction. J Biol Chem 1998;273:33893–6.
[150] Aoki N, Matsuda T. A cytosolic protein tyrosine phosphatase PTP1B specifically dephosphorylates and deactivates prolactin-activated STAT5a and STAT5b. J Biol Chem 2000.
[151] Klingmueller U, Lorenz U, Cantley LC, Neel BG, Lodish HF. Specefic recruitment of SH-PTP1 to the erythropoietin receptor causes inactivation of Jak2 and termination of proliferative signals. Cell 1995;80:729–38.
[152] Vinkemeier U. Getting the message across, STAT! Design principles of a molecular signaling circuit. J Cell Biol 2004;167:197–201.
[153] ten Hoeve J, de Jesus Ibarra-Sanchez M, Fu Y, Zhu W, Tremblay M, David M, et al. Identification of a nuclear Stat1 protein tyrosine phosphatase. Mol Cell Biol 2002;22:5662–8.
[154] Croker BA, Kiu H, Nicholson SE. SOCS regulation of the JAK/STAT signalling pathway. Semin Cell Dev Biol 2008;19:414–22.
[155] Müller P, Boutros M, Zeidler MP. Identification of JAK/STAT pathway regulators—insights from RNAi screens. Semin Cell Dev Biol 2008;19:360–9.
[156] Groner B, Lucks P, Borghouts C. The function of Stat3 in tumor cells and their microenvironment. Semin Cell Dev Biol 2008;19:341–50.
[157] Wilks AF. The JAK kinases: not just another kinase drug discovery target. Semin Cell Dev Biol 2008;19:319–28.
[158] Vainchenker W, Dusa A, Constantinescu SN. JAKs in pathology: role of Janus kinases in hematopoietic malignancies and immunodeficiencies. Semin Cell Dev Biol 2008;19:385–93.