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Interferons as a paradigm for cytokine signal transduction

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Abstract. Characterization of the ability of interferons to induce immediate early genes led to the identification of the signal transducer and activators of transcription (STAT) signaling paradigm, where a single protein transduces signals directly from the receptor to the nucleus. Subsequent studies have determined that all cytokines transduce pivotal signals through at least one of the seven members of this STAT family. Notably, cytokines can be placed in functional subgroups based on the STATs they activate.

Key words. Interferons; cytokines; STATs; JAKs; signal transduction.

Introduction

In the late 1970s and early 1980s there was much interest in identifying molecules that regulate transcription. Regulatory DNA elements, and the proteins they bound were being identified in the promoters of many eukaryotic genes. This period also witnessed the production of interferon (IFN) as one of the first products of the nascent biotechnology industry. The availability of relatively large quantities of pure IFN provided an opportunity to determine if and how peptide-based ligands regulate gene expression. Genes were identified whose expression was upregulated in response to treatment with recombinant type I IFNs [1, 2]. Molecular characterization of the ability of these proteins to induce genes led to the identification of the first two members of the signal transducer and activators of transcription (STAT) family of signaling proteins [3, 4]. Subsequent studies determined that IFN-γ also transduced signals through a STAT [5]. These biochemical studies were complemented by a genetic experiment that led to the identification of TYK2 [6], a member of the Janus (i.e., JAK) family of soluble tyrosine kinases. More recent studies have determined that all cytokines, as well as other classes of ligands, transduce signals through the sequential activation of JAKs and STATs (see table 1) [7–9].

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The type I IFN signaling paradigm

IFNs constitute an important component of innate and acquired immune responses. In addition to being ascribed key roles in host defense against viral and parasitic infections, they exhibit antiproliferative and tumoricidal activity [10, 11]. IFNs can be divided into two major classes, type I and type II. Type I IFNs consist of a group of over 20 functionally and structurally related ligands, including all of the IFN-αs (by far the largest group), IFN-β, IFN-ω, and IFN-τ. They all bind and transduce signals through the same (i.e., type I) IFN receptor [12]. IFN-γ, or immune IFN, is the only member of the type II family, and it signals through a distinct receptor.

Type I IFNs play a more critical role in innate and early host response to viral infection. The potent antiviral responses induced by IFNs in target cells led to an early interest in developing them as therapeutic agents and characterizing the nature of their biological response. Identification of several INF-α-inducible genes suggested that the expression of new genes was likely to be important in the biological response to these and other potential ligands. These genes included those known to mediate potent antiviral activities [e.g., protein kinase R (PKR), 2′-5′ oligodenylate synthetase, and MX; refs 13–15], as well as those whose functions were less understood [e.g., IFN-stimulated gene (SIG)15, ISG54, 6–16; refs. 16–18].

Careful characterization of the promoters of ISG15 and ISG54 led to the identification of a critical IFN-α-responsive enhancer, the ISRE [16, 17]. Subsequent studies identified several factors that bound this element directly. The ISRE-binding activity of one of these factors, IFN-gene-inducible factor 3 (ISGF3), correlated directly with transcriptional induction [19]. The most effective ISRE (CAGTTTCGGTTTCCC) was found in the ISG15 promoter [17]. Compilation of several functionally characterized ISREs yielded the consensus sequence, YAGTTTC(A/T)YTTTYCCC [19]. Detailed mutagenesis studies both confirmed this consensus and demonstrated that at the core of the ISRE is an interferon-responsive transcription factor (IRF)-1-binding element, TTTCGGTTTC (actually two tandem binding sites [20, 21]).

Table 1. Summary of JAK and STAT usage by extracellular ligands (see text for details) (IL, interleukin; G, granulocyte; CSF, colony-stimulating factor; GM, granulocyte-macrophage).

Ligands	JAK	STATs
IFN family		
IFN-α/β	TYK2, JAK1	STAT1, STAT2, STAT3, STAT5
IFN-γ	JAK1, JAK2	STAT1, STAT5
IL-10	TYK2, JAK1	STAT1, STAT3
Gp130 family		
IL-6	TYK2, JAK1, JAK2	STAT3, STAT1
IL-11	JAK1	STAT3, STAT1
Oncostatin M	JAK1, JAK2	STAT3, STAT1
Leukemia inhibitor factor	TYK2, JAK1, JAK2	STAT3, STAT1
Colony neurotrophic factor	TYK2, JAK1, JAK2	STAT3, STAT1
G-CSF	JAK1, JAK2	STAT3, STAT1
IL-12	TYK2, JAK2	STAT4
Leptin	JAK2	STAT3
γ-C family		
IL-2	JAK1, JAK2, JAK3	STAT3, STAT5
IL-4	JAK1, JAK3	STAT6
IL-7	JAK1, JAK3	STAT3, STAT5
IL-9	JAK1, JAK3	STAT1, STAT3, STAT5
IL-13	JAK1	STAT6
IL-15	JAK1, JAK3	STAT3, STAT5
IL-3 family		
IL-3	JAK2	STAT5
IL-5	JAK2	STAT5
GM-CSF	JAK2	STAT5
Single-chain family		
Erythropoietin	JAK2	STAT5
Growth hormone	JAK2	STAT1, STAT3, STAT5
Prolactin	JAK2	STAT5
Thrombopoietin	JAK2, TYK2	STAT5
Receptor tyrosine kinases		
Epidermal growth factor	JAK2, JAK2	STAT1, STAT3, STAT5
Platelet-derived growth factor	TYK2, JAK1, JAK2	STAT1, STAT3
CSF-1	TYK2, JAK1	STAT1, STAT3, STAT5
G-protein-coupled receptors		
AT1	TYK2, JAK2	STAT1, STAT2

Detailed characterization of ISGF3 determined that it consisted of two biochemically distinct components. The cytosolic α-component, which became activated upon stimulation with ligand, and the γ-component, which had modest intrinsic DNA-binding activity [19, 22]. Biochemical purification identified the γ-component as a single 48-kDa protein and a member of the IRF-1 family of transcription factors [3, 23]. A similar analysis of the α-component determined that it consisted of three proteins of 113, 91, and 84 kDa [3]. p84 and p91 were identified as alternative splice isoforms of a novel gene, named STAT1. p113 was determined to be encoded by a related but distinct gene, STAT2 [4]. Subsequent DNA-binding studies have confirmed that p48 interacts with the 'IRF-1 core' and that the STAT1:STAT2 heterodimer both enhances the DNA-binding affinity and defines the specificity of binding through interactions with nucleotides flanking the core [24].

Once biochemically characterized, it became possible to determine how the components of ISGF3 transduce signals. Metabolic labeling studies demonstrated that both STAT1 and STAT2 became activated by a single tyrosine phosphorylation event [5]. Moreover, they demonstrated that this activation event rendered these proteins competent for association, yielding an active α-component. The phosphotyrosines were mapped to residue 701 for STAT1 and 691 for STAT2 [25, 26]. They were found to mediate dimerization through a reciprocal interaction with the SH2 domain of the corresponding STAT partner [27, 28]. Immunofluorescence studies demonstrated that while the expression of inactive STAT1 and STAT2 was restricted to the cytosolic compartment, activated proteins rapidly translocated to the nucleus [5].

Another approach to identify the components of the IFN-α signaling cascade entailed the development of a set of IFN-resistant cell lines [29]. Several rounds of mutagenesis led to the generation of six distinct complementation groups [30–32]. Characterization of the first complementation group determined that it represented a defect in TYK2, the founding member of the JAK family of soluble tyrosine kinases [6], the other members of which are JAK1, JAK2 and JAK3 [8]. This observation implicated members of the JAK family as the elusive tyrosine kinases that were required for signaling by the catalytically inactive cytokine receptor family. It also suggested that TYK2 might mediate the IFN-α-dependent activation of STAT1 and STAT2.

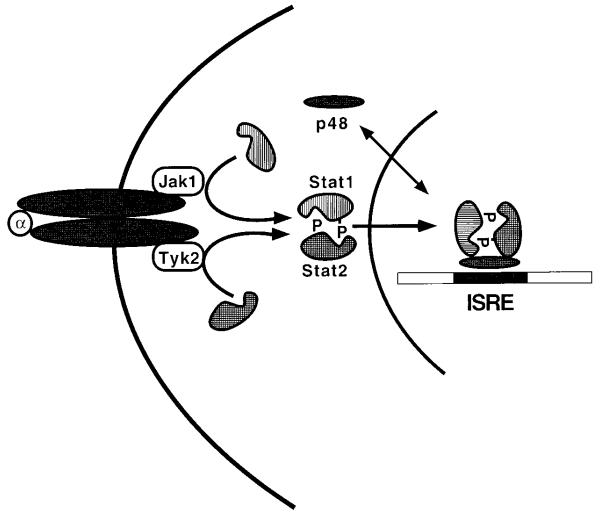

Figure 1. The IFN-α-stimulated JAK-STAT pathway. IFN-α stimulates the activation of two receptor-associated tyrosine kinase, JAK1 and TYK2. These kinases in turn mediate the activation of STAT1 and STAT2. Activated STAT1 and STAT2 are released from the receptor and associate into stable heterodimers. The STAT1:STAT2 heterodimers translocate to the nucleus, where they associate with p48 to form a stable complex on the ISRE, promoting the induction of target genes. The STAT SH2 domain, illustrated by a concave arc, plays an important role for several steps in the signaling cascade. See text for details.

Subsequent studies demonstrated that the defects in the three remaining complementation groups could be rescued by p48, STAT1, and STAT2 cDNAs [23, 31–34]. U3 cells, which were found to be STAT1 deficient, were important in establishing that both isoforms of STAT1 (i.e., p84 and p91) rendered the α-component fully functional in IFN-α signaling [33]. STAT2-deficient U6 cells were important for establishing that the carboxy terminus of STAT2 encoded the transcriptional activation domain required for ISGF3 activity [35].

The IFN signaling defects in the two remaining mutant lines (U4 and U5) were shown to be rescued by the introduction of JAK1 and IFNAR2 (the β-chain of the type I IFN receptor) [36, 37]. Subsequent studies demonstrated that TYK2 and JAK1 functionally associate with membrane proximal domains in the α- and β-chains of the type I IFN receptor [38, 39]. Activated forms of these kinases then mediate the activation of STAT1 and STAT2. This signaling paradigm, highlighting the IFN-α-dependent activation, dimerization, nuclear translocation, and DNA binding of STAT1 and STAT2, is outlined in figure 1.

The type II IFN signaling paradigm

IFN-γ is a potent immunomodulatory cytokine. In contrast to type I IFNs, it plays a vital role in acquired immune responses, and in some innate immune responses [40]. Like type I IFNs, IFN-γ achieves these effects through the induction of a number of immediate early genes. Characterization of the ability of IFN-γ to induce one of these genes, guanylate-binding protein (GBP), led to the identification of the first IFN-γ activation site (GAS) and the factor it bound, the gamma activation factor (GAF) [41, 42]. The identification of additional and more robust IFN-γ-responsive elements [43, 44] permitted elucidation of the GAS consensus element, TTTCCNGGAAA. Of note, concurrent studies characterizing the ability of other ligands [e.g., platelet-derived growth factor (PDGF), interleukin (IL)-6, and prolactin (PRL)] to induce immediate early genes led to the identification of response elements that fit the GAS consensus [45–47].

Taking advantage of the reagents that had been generated against components of ISGF3, it quickly became apparent that STAT1 was an essential component of GAF [48]. Moreover, IFN-γ mediated the same STAT1 tyrosine phosphorylation event previously found with IFN-α. Consistent with the palindromic nature of the GAS element, biochemical studies demonstrated that GAF consisted of a STAT1 homodimer.

The realization that STAT1 was required for the biological response to both type I and type II IFNs provided a simple explanation as to why U3 cells, selected for their failure to respond to IFN-α, also failed to respond to IFN-γ [33]. There was however one important difference. Whereas both full-length STAT1 (i.e., p91) and its carboxy terminally truncated isoforms (i.e., p84) restored the ability of these cells to respond to type I IFNs, only p91 rescued the response to IFN-γ. The simulation of U3-p84-complemented cells with IFN-γ did, however, led to the nuclear accumulation of an active (i.e., DNA-binding) p84 complex. But this complex was transcriptionally inert, leading to the conclusion that the missing carboxy terminus must encode a transcriptional activation domain (TAD) of STAT1 (not required for ISGF3 function).

Characterization of two additional mutants, U4 and γ1, provided additional important insight into the IFN-γ signaling paradigm. The rescue of IFN-γ signaling in the U4 mutants by the introduction of JAK1, and in the γ1 mutants by the introduction of JAK2 implicated both kinases in IFN-γ signaling. This was further supported by biochemical studies demonstrating that these two kinases are activated by IFN-γ [49], and those indicating that JAK1 and JAK2 associate with the membrane proximal region of the α and β IFN-γ receptor chains [50–53].

Characterization of the α-chain of the IFN-γ receptor led to the identification of domains that were critical to signaling [54, 55]. The proximal domain was found to mediate association with/activation of JAK1 [53]. The second domain, centered around tyrosine 400 (Y440), became tyrosine phosphorylated in response to ligand. Moreover, the SH2 domain of STAT1 bound the phosphorylated Y440 avidly. This indicated that receptor activation itself was responsible for the recruitment of STAT1 to the receptor complex [56]. Consistent with other SH2 domain-phosphotyrosine interactions, residues carboxy terminal to Y440 were found to play an important role in defining the specificity of this interaction [57]. Studies demonstrating that mutations in the STAT1 SH2 domain crippled activation provided additional support for this model [58]. In summary, studies on IFN-γ-stimulated gene induction have yielded a well-defined signaling paradigm (see fig. 2), which is now known to be employed by all other cytokines.

The STAT family

STAT1 and STAT2 were initially identified as the proteins that mediate the IFN-specific induction of a specific set of immediate early genes. Five additional STATs have now been identified either through homology in sequence, or symmetry in signaling. Analogous to the IFN-γ signaling paradigm, these additional STATs all bind to GAS elements. The biological importance of the STAT signaling paradigms has come to light through careful biochemical and genetic studies (table 1; and see contribution by D. E. Levy).

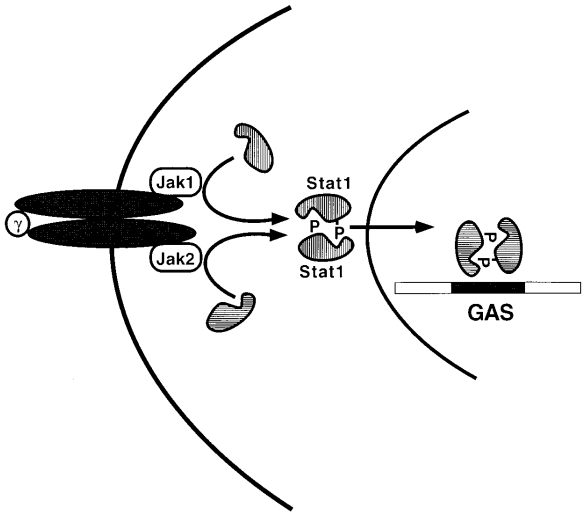

Figure 2. The IFN-γ-stimulated JAK-STAT pathway. IFN-γ stimulates the activation of two receptor-associated tyrosine kinases, JAK1 and JAK2. These kinases promote the sequential activation of the receptor and STAT1. Activated STAT1 is released from the receptor whereupon it forms homodimers. These homodimers translocate into the nucleus, where they bind to the GAS element directly and promote the induction of target genes. The STAT SH2 domain, illustrated by a concave arc, plays an important role for several steps in the signaling cascade. See text for details.

STAT family members

Studies primarily directed at determining how members of the cytokine family transduce signals have led to the identification of seven mammalian STATs, including STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6.

STAT1. The purification of STAT1 and STAT2 as the critical components of IFN-stimulated transcription factors has provided compelling evidence for their role in mediating the biological response to IFNs [3, 5]. However, subsequent studies determined that members of the IL-6 family of cytokines and epidermal growth factor (EGF) activate STAT1 along with STAT3 [59–63]. As STAT1-specific antibody reagents have become more widely available, additional STAT1-activating ligands have been reported [9]. However, STAT1 gene-targeting studies, outlined in the review by D. E. Levy, suggest SAT1 is relatively specific for type I and type II IFNs [64, 65].

STAT2. STAT2 was first identified as a critical component of the type I IFN signaling cascade [3–5]. In contrast to other STATs, there is little compelling evidence that STAT2 plays an important role in any other signaling cascade. Moreover, recent gene-targeting studies appear to support this conclusion [D. E. Levy in this issue; 65a]. STAT2 exhibits a number of other features that set it apart from other STATs. For example, even though it contains each of the major STAT domains (see below), there is little evidence that active STAT2 homodimers form in vivo. In addition, there is a remarkable amount of divergence between the human and murine isoforms [C. Park and C. Schindler, unpublished data]. Whereas the murine homologues of other STATs exhibit 85–90% homology, murine STAT2 is only $\sim$75% similar to human STAT2 in its first 700 amino acids. After that point, the sequences are completely divergent. Nevertheless, these two homologues appear to be functionally quite conserved.

STAT 3. Biochemical studies have implicated STAT3 in signaling for a large number of cytokines, including all members of the IL-6 family [63, 66–69]. Even though STAT1 appears to be co-activated with STAT3, genetic studies indicate that STAT3 is by far the most critical for signal transduction. For example, dominant

interfering mutants of STAT3, but not STAT1, block the ability of IL-6 to induce differentiation in M1 cells [70, 71], or to induce IgM production in SKW6.4 cells [72]. More physiological studies in primary neural cultures have demonstrated that dominant interfering STAT3 mutants block gliogenesis induced by ciliary neurotrophic factor [73]. Likewise, a dominant interfering STAT3 mutant blocks leukemia inhibitory factor (LIF)-dependent self-renewal in pluripotent ES cells [74, 74a]. Consistent with a potential role for STAT3 in proliferation, several studies have implicated STAT3 in transformation by *v-abl* and *v-src* [75–80]. More recently, *c-myc* has been identified as a target gene for STAT3 in IL-6-stimulated cells, providing a potential explanation for the transforming potential of this STAT [81]. Other studies suggest that STAT3 may play a more important role in an IL-6-mediated prevention of apoptosis [82–84]. STAT3 has also been strongly implicated in IL-10 signal transduction by genetic studies [85, 85a].

STAT4. STAT4 was initially identified through a low-stringency screen [66]. It remained an orphan STAT for almost 2 more years until it was determined that IL-12 stimulated its activation [86]. Gene-targeting studies (see review by D. E. Levy) have largely confirmed these biochemical observations. Of note, despite the high level of STAT4 expression in testes, spermatogenesis is normal in STAT4 knockout mice [87].

STAT5a and STAT5b. STAT5a and STAT5b are encoded by two highly homologous genes ($\sim 96\%$ amino acid identity) [88–90]. They diverge modestly at their carboxy termini and are functionally redundant in some, but not all, assays [89, 91, 92]. STAT5a and STAT5b were initially identified during the purification of PRL- and IL-3-stimulated transcription factors [46, 88]. Subsequent studies determined that all members of the single-chain receptor family [i.e., growth hormone (GH), PRL, erythropoietin (EPO), and thrombopoietin (TPO)], the IL-2 receptor family (i.e., IL-2, IL-7, IL-9, and IL-15) and the IL-3 receptor family [i.e., IL-3, IL-5, granulocyte-macrophage-colony stimulating factor (GM-CSF)] mediate the activation of STAT5 [89, 93–97]. Importantly, several STAT5 target genes have been identified in cells stimulated with these ligands [46, 98–101]. Studies have also implicated STAT5 in the development and activation of specialized myeloid lineages [102–106]. More recently, carboxy terminally truncated isoforms of STAT5 have been found to be preferentially expressed in myeloid progenitors [88, 103, 104, 107]. These isoforms do not mediate the activation of known STAT5 target genes, suggesting they may enable these cells to exhibit a biologically distinct response. In contrast to other carboxy terminally truncated STAT isoforms, the truncated STAT5 isoforms are generated through the activity of a lineage-specific protease [108]. Gene-targeting studies, detailed in this issue by D. E. Levy, indicate that STAT5a and STAT5b are particularly important in PRL and GH function [109–111].

STAT6. As with STAT4, biochemical and genetic studies have implicated STAT6 as a signal transducer for a single cytokine, IL-4 and the closely related IL-13 [112–117]. IL-4 plays an important role in the development of a subset of T helper cells (i.e., Th2 cells) and some aspects of B cell maturation [118]. Hence, a balance between STAT4 and STAT6 signaling pathways must be achieved to obtain an appropriate ratio of Th1- and Th2-dependent responses (i.e., cellular vs humoral immune responses; see Mui in this issue).

STAT genetic loci. The genetic loci of all seven murine STAT genes have been determined [119]. STAT1 and STAT4 co-segregate to the proximal region of chromosome 1. STAT2 and STAT6 also co-segregate, and map to distal chromosome 10. The final cluster includes the genes for STAT3, STAT5a and STAT5b. They co-segregate to distal chromosome 11. Of these, the STAT5a and STAT5b genes are the most tightly linked [111]. Based on these mapping studies, the location of the human genes can be predicted. Hence, human STAT1 and STAT4 genes are likely to map to human chromosome 2 (2q12–2q33). Human STAT2 and STAT6 genes are likely to map to human chromosome 12 (12q13–12q14.1). And finally, human STAT3, STAT5a, and STAT5b genes are likely to map to human chromosome 17 (17q11.2–17q22). A model to account for this distribution of the STAT family of genes would suggest that a primordial STAT gene (perhaps most closely related to STAT5; see C. R. Dearolf in this issue), was tandemly duplicated. This event was then followed by two additional larger duplications of the tandem STAT locus. Finally, the STAT5 gene underwent one final duplication, leading to three STAT genes at this one locus.

STAT structure

STATs have been identified in mammals and several lower eukaryotes including *Drosphila*, *Caenorhabditis elegans*, and *Dictyostelium* (the contribution by C. R. Dearolf). With a few exceptions, these STATs share several structurally and functionally conserved domains (see fig. 3) [120–122], including an amino terminal domain (NH_2), a coiled-coil domain, a DNA-binding domain (DBD), an SH2 domain, and a tyrosine activation motif (Y). The sequences of the carboxy terminal TADs are not well conserved. The recent crystal structures of STAT1 and STAT3 (bound to DNA) have more clearly defined these domains (see fig. 4) [121, 122].

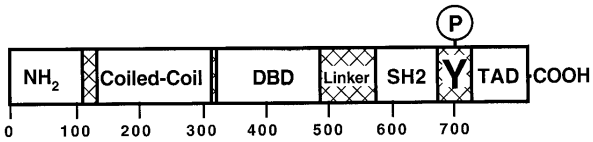

Figure 3. STAT structure. STATs share a number of conserved domains. These include an amino-terminal domain (NH_2), a coiled-coil domain, a DNA-binding domain (DBD), a linker domain, an SH2 domain, and a tyrosine activation motif (Y). The sequences carboxy terminal to this tyrosine encode a transcriptional activation domain (TAD), but are not conserved. See text for details.

Amino terminus. The amino-terminal domain spans ~ 125 amino acids and is one of the most conserved STAT domains. However, it can be removed from the core STAT complex by limited proteolysis, indicating that it is a physically distinct domain [120]. Structural and functional studies have provided compelling evidence that this domain mediates a co-operativity in DNA binding when there are tandem GAS elements [120, 123]. The crystal structure of the amino terminus of STAT4 indicates that it achieves co-operativity by promoting an interaction between two STAT amino termini [124]. STAT amino termini have also been implicated in other functions. For example, some studies suggest that the amino terminus of STAT2 may promote interactions with the β-chain of the IFN-α receptor [31]. Other studies suggest that the amino terminus of STAT1 may promote association with p300/CBP [125]. Additionally, mutagenesis and deletional studies suggest that the amino terminus may participate in the regulation of nuclear translocation [126]. Amino-terminal STAT mutants also exhibit a defect in deactivation (i.e., signal decay), indicating that nuclear translocation and deactivation may be causally linked [126, 127].

Coiled-coil domain. The STAT crystal structures have identified a coiled-coil domain, or four-helix bundle, extending from amino acids ~ 135 to ~ 315 [121, 122]. The pair of four-helix bundles protrude laterally (~ 80 Å) from the core, supporting the notion that they mediate interaction with other proteins. One protein with which this domain is likely to associate is p48, the DNA-binding component of ISGF3 [128, 129]. Yeast two-hybrid assays, employing this domain as bait, have yielded several interacting proteins including PIAS, Nmi, and StIP1 [130–132; R. Collum et al., unpublished data]. These proteins all seem to modulate (i.e., potentiate or antagonize) the activity of STATs.

DNA-binding domain. Studies based on both natural and optimal STAT DNA-binding sites indicate that the consensus element for all STAT homodimers (and some heterodimers) is $TTCN_{2-4}GAA$. Some STAT dimers exhibit a preference of $N = 2$, others for $N = 3$ or 4 [133–137]. The spacing between palindrome half-sites is an important determinant in defining STAT binding specificity [138]. The STAT DBD was first mapped to mid-molecule through the generation of STAT1/STAT3 chimeras [134]. These studies took advantage of the modest difference in DNA-binding affinity between these two STATs. The crystal structures confirm these observations and demonstrate that the DBD (amino acids ~ 320–475) includes a β-barrel and fits the general architecture of an immunoglobulin fold [121, 122]. As anticipated, amino acids important in the recognition of the GAS element are highly conserved. The STAT structures also indicate that DNA binding is critically dependent both on the dimerization domains (see fig. 4 and below) and linker sequences separating these domains. Of additional note, STAT2, which is unable to bind GAS elements directly, exhibits a high degree of conservation in each of the regions important for DNA binding.

SH2 domain. SH2 domains and tyrosine phosphorylation appear to have co-evolved in metazoans [139]. The SH2 domain of the STAT proteins in *Dictyostelium* may represent one of the 'earliest' SH2 domains [140]. Of note, the STAT SH2 domain is remarkably divergent in sequence, but not structure, from the prototypical v-Src SH2 domain. Could the STAT SH2 domain represent the primordial SH2 domain (see Dearolf in this issue)? Functionally, the STAT SH2 domain (amino acids ~ 585–685) has been shown to play an important role in three aspects of signaling. First, it defines the specificity of STAT recruitment to the appropriate receptor [56, 58, 97, 141]. Second, it mediates an obligate interaction with the activating JAK [28, 142]. Third, it mediates STAT dimerization, which is achieved by the reciprocal/symmetric and specific interaction between the SH2 domain of one partner and the phosphotyrosine of the other partner [27, 28]. Consistent with its critical role in STAT signaling, this SH2 domain is the most highly conserved STAT domain [121, 122]. The STAT crystal structure confirms the role of this domain in mediating a reciprocal dimerization event (see fig. 4). Moreover, it demonstrates, as is the case for other SH2 domains, that the interaction with the ligand is limited to a phosphotyrosine plus adjacent carboxy-terminal residues. In the case of STAT1, for which there is better structural detail, the phosphotyrosyl residue (i.e., Y701), along with seven carboxy-terminal residues, mediate this interaction. Residues at $+1$, $+3$, and $+5$ appear to be particularly important. The variability in both size and chemical composition of this region among members of the STAT family provides further evidence that this interaction is likely to account for a great deal of the specificity in STAT receptor binding and STAT dimerization.

Tyrosine activation motif. Initial studies characterizing STAT1 and STAT2 determined that they were activated by a single tyrosine phosphorylation event. This tyrosine and the surrounding residues are referred to as the tyrosine activation motif and have been mapped near residue 700 for each of the seven mammalian STATs [121, 122]. As detailed in the preceding section, the interaction between the SH2 domain and this tyrosine is critical to several steps in STAT signal transduction. The proximity of this tyrosine to the corresponding SH2 domain (i.e., $\sim 10-15$ amino acids carboxy terminal) effectively prevents it from interacting with its own SH2 domain [27, 121, 122]. The sequences distal to the activation tyrosine (i.e., positions $+1$ to $+7$) are highly variable between STAT family members, contributing to the high degree of specificity in STAT receptor recruitment and dimerization [57]. While each STAT, except STAT2, can homodimerize, only a limited number of STAT heterodimers have been identified, even under conditions where STATs are artificially overexpressed [7, 9]. There is compelling evidence that STAT1:STAT3 and STAT5a:STAT5b heterodimers form in vivo [89, 143]. Additional heterodimers have been reported, but their existence is less well documented [144, 145].

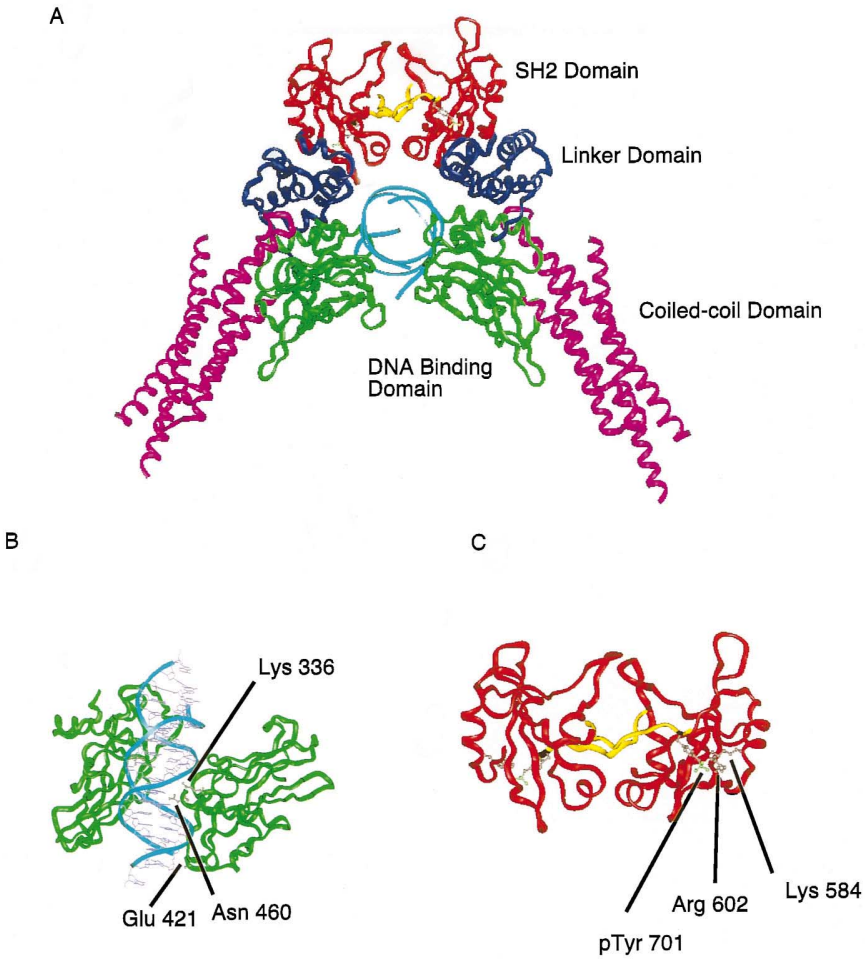

Figure 4. Ribbon diagrams of a STAT1 dimer bound to DNA. These diagrams were generated by the Insight II (Silicon Graphics) with coordinates deposited at http://www.rockefeller.edu/kuriyan/ [121]. (*A*) The STAT1 homodimer (amino acids 136–710) complexed to an 18-bp oligonucleotide. The coiled-coil domain (amino acids 136–317) is shown in pink, the DNA-binding domain (amino acids 318–488) is shown in green, the linker domain (amino acids 489–576) is shown in purple, and the SH2 domain (amino acids 577–683) is shown in red. The flexible arm of the SH2 domain that includes phosphotyrosine 701 is shown in yellow. (*B*) Detailed view of the STAT1 DNA-binding domain (amino acids 318–488). The side chains of Lys336, Glu420, and Asn460, which are thought to make direct or water-mediated contact with the DNA, are shown. (*C*) Detailed view of the STAT1 SH2 dimerization domain. The side chains of Lys584 and Arg602, which are crucial for the interaction with pTyr701, are shown.

Carboxy terminus. The carboxy terminus is defined as all sequences carboxy terminal to the tyrosine activation motif. It is the most divergent STAT domain. The possibility that this region might encode a TAD was first suggested by the distinct properties of the full-length (i.e., p91) and carboxy terminally truncated (i.e., p84) isoforms of STAT1 (see above) [33]. In subsequent studies, TADs have been mapped to the carboxy terminus of each mammalian STAT [108, 137, 146–150; C. Park and C. Schindler, unpublished data]. Both deletion and 'classic' GAL4 fusion assays [151] suggest that TADs vary significantly in potency and reside in the most carboxy terminal ~ 50 amino acids [148; C. Park and C. Schindler, unpublished data]. These observations raise the question as to the function of the remaining carboxy-terminal residues (i.e., more amino terminal), especially in STAT2 and STAT6 [7]. Intriguingly, in contrast to all other STATs, the carboxy-terminal sequence of STAT2 is not conserved between human and mouse, even though they appear to be functionally conserved [65a].

TADs also appear to be regulated by posttranslational modifications, which include serine phosphorylation (see the contribution by Decker and Kovarik) and truncations. As predicted, when carboxy terminally truncated isoforms are 'artificially' overexpressed, they antagonize signaling [70, 71, 146, 149, 152, 153]. However, there is little compelling evidence that 'naturally occurring' truncated STATs exhibit a dominant negative phenotype. Rather, they appear to play a more positive role. For example, the carboxy terminally truncated isoforms of STAT1 (i.e., p84), which is generated by RNA processing, is fully functional in type I, but not in type II IFN signaling [33]. p84 achieves its positive role in type I IFN signaling by associating with another transcription factor, STAT2 [5]. Likewise, the carboxy terminally truncated isoform of STAT3, also generated by RNA processing, appears to play a positive role in signaling by associating with the transcription factor c-Jun [154, 155]. Preliminary studies suggest that the carboxy terminally truncated isoforms of STAT5, which are generated by a novel proteolytic process in myeloid progenitors, may also play a positive role [104, 107, 108, 156]. Hence, regulating the type of STAT isoform expressed (i.e., full length vs truncated) may afford the cell an opportunity to mount lineage-specific biological responses to the same ligand.

STAT nuclear translocation

The rapid translocation of STATs from the cytoplasm to the nucleus after ligand-dependent activation is critical to the JAK-STAT signaling paradigm. However, the mechanism by which this is achieved has not been fully elucidated. Based on the substantial progress that has been made in determining how other proteins are transported to the nucleus, it has been possible to begin to outline the process by which STATs translocate into the nucleus.

Many large proteins (i.e., > 50 kDa) that translocate into the nuclear compartment carry a tag or nuclear localization sequence (NLS). This tag is recognized by components of the nuclear transport machinery [157]. The 'classic' NLS consists either of a short stretch of continuous basic amino acids (e.g., the SV40 T antigen NLS) or of two short and moderately separated stretches of basic amino acids. The classic NLS sequences are recognized by the α-subunit of a transport protein, referred to as α-importin or α-karyopherin This α-subunit can bind to the β-subunit, referred to as β-importin or $\beta 1$-karyopherin. This subunit stably associates with the Ran-GTPase complex at the nuclear pore complex. Ran-GTPase plays a critical role in assuring vectoral translocation through this portal into the nucleus. A second class of NLSs, comprising a conserved 38-amino-acid sequence (known as M9), has also been characterized. M9 was identified on heterogeneous ribonuclear protein A1, and regulates both nuclear import and export. It achieves this by directly associating with another member of the karyopherin family, $\beta 2$. Likewise, this mode or translocation though the nuclear pore is energy (probably Ran) dependent, but $\beta 1$ independent. Recently, additional karyopherins have been identified. Of note, many proteins are also actively exported from the nucleus. Nuclear export sequences (NES) that mediate this process have in some cases been shown to associate with karyopherins (i.e., those that have already been translocated into the nucleus).

Recent studies suggest that the ability of STAT1 to translocate to the nucleus is dependent on Ran-GTPase activity [158], implicating karyopherins in this process as well. It has also been determined that activated STAT1 will only bind to one of the two major classes of α-karyopherins [159]. This contrasts with the SV40 T antigen, whose NLS is able to bind both of these types of α-karyopherin. The STAT1-α-karyopherin complex subsequently associates with $\beta 1$-karyopherin, suggesting that STATs may employ the 'classical-like' NLS pathway for nuclear translocation. However, attempts to identify the STAT NLS have been unsuccessful [159; I. Strehlow and C. Schindler, unpublished observations].

Another important issue for STATs is how activation (i.e., tyrosine phosphorylation) triggers nuclear translocation. Other transcription factors are known to be tethered in the cytoplasm by anchoring protein. Activation leads to a phosphorylation event and release from the anchoring protein [160, 161]. However, these

proteins differ from STATs in one important aspect: they are not activated by the receptor. Rather, they become activated while tethered to a cytoplasmic anchoring protein. These observations suggest it may be necessary to regulate STAT sublocalization by a different mechanism. Perhaps the ability of STATs to dimerize after activation presents an NLS that may either span both peptide components of the dimer, or is conformationally obscured. Consistent with these models, recent studies have demonstrated that dimerization will rescue the ability of amino terminally mutant STATs to translocate to the nucleus [126]. Although these observations fail to distinguish between two possibilities, they do provisionally map the STAT NLS to the amino terminus.

Another potentially important observation regarding the regulation of STAT nuclear translocation is that there appears to be a basal level of STAT1 and STAT3 in the nucleus of many unstimulated cells [5, 162]. While this basal nuclear staining has often been attributed to stimulation by an unknown ligand (i.e., present in culture media), recent studies suggest that a basal level of nuclear STATs may be required to regulate capase expression [163, 164]. This will be more fully discussed in the following section.

'Nonclassical' STAT functions

Most cytokines have been shown to transduce signals through the 'classical' STAT signaling paradigm (see figs. 1, 2). However, several recent observations are difficult to reconcile with this model and suggest that STATs may exhibit some additional 'nonclassical' functions. These include networking (i.e., interacting) with other signaling cascades, as well as regulating apoptosis.

Networking with other pathways

As discussed elsewhere in this issue by Decker and Kovarik, the requirement of serine phosphorylation for full STAT activity indicates that STATs can integrate signals from other pathways, i.e., those that regulate serine phosphorylation. Recently, several additional examples of how STATs may network with other pathways have been reported, including interactions with PKR and phosphatidylinositol 3-kinase (PI3K) [165, 166].

STAT-PKR interaction. PKR is a serine/threonine-specific protein kinase that is important in antiviral responses. Its expression is induced by both IFNs and double-standard RNA (dsRNA, i.e., viral RNA). PKR becomes fully active after exposure to dsRNA, whereupon it phosphorylates the α-subunit of the eukaryotic initiation factor 2 (eIF-2α). This effectively blocks translocation of both endogenous and viral genes [167]. Recently, PKR has also been implicated in STAT1-mediated transcriptional regulation [165]. It achieves this by associating with unphosphorylated STAT1 to yield an inactive PKR-STAT1 complex. Upon stimulation with either IFN or dsRNA, STAT1 is released, increasing the pool available for activation. The potential role of this interaction during viral infection, or the ability of PKR to bind other STATs, remains to be explored.

STATs as a docking molecule. In contrast to IFN-γ, type I IFNs have been shown to activate several signaling cascades. This certainly includes two JAK-STAT pathways, the ISGF3 $\rightarrow$ ISRE pathway and the STAT1:1 $\rightarrow$ GAS pathway. In many cell types, STAT3 appears to be activated as well, leading to the formation of STAT3:3 homodimers and STAT3:1 heterodimers [60, 134]. With the identification of a potential YXXQ STAT3 recruitment motif in IFNARI [168], a role for STAT3 in the biological response to IFNs has been suggested [169]. In addition, type I IFNs have been shown to induce the PI3K pathway. Recent studies exploring the IFN-α-mediated activation of STAT3 and PI3K have led to the proposal that STAT3 serves as a docking molecule to recruit the p85 subunit of PI3K to the IFN-α receptor complex [166]. This model suggests that a novel phosphotyrosine (i.e., Y656, not the activation tyrosine) on receptor-associated STAT3 is bound by the SH2 domain of p85. However, it remains to be determined whether Y656 is sufficiently exposed to serve as a docking site or whether it actually becomes phosphorylated.

STATs and apoptosis

Cytokines appear to contribute to the growth and maturation of several hematopoietic lineages. It has been suggested that they achieve this either through the stimulation of cell cycle progression or possibly the suppression of apoptosis [70, 71, 81–83, 170, 171]. When cells undergo terminal differentiation, proliferation is also suppressed. STATs may participate in this process by inducing genes encoding cyclin inhibitors [172–174]. STATs may also upregulate the expression of genes involved in programmed cell death, but this may be independent of any ligand [163]. Studies evaluating cell death in IFN-resistant cell lines have determined that STAT1-deficient U3 cells are uniquely resistant to tumor-necrosis-factor-induced apoptosis. This defect correlates with a loss in the basal expression of three caspases (proteases implicated in programmed cell death) and can be rescued by STAT1 cDNAs that are defective in 'classical' JAK-STAT signaling (e.g., R602L, Y701F, and S727A). These observations suggest that STAT1 can regulate the expression of caspases without forming active STAT1 homodimers. How? Per-

haps STAT1, which is found at low levels in the nucleus of unstimulated cells [5, 162], associates with other proteins to regulate the expression of caspases [164]. This should be an intriguing area for future investigation.

Concluding comments

Initially identified as components of IFN-stimulated transcription factors, seven STATs have now been identified. They are related both structurally and functionally. Biochemical and genetic studies have provided compelling evidence that these STATs transduce signals critical to the biological response of most, if not all, cytokines. Many of these responses may have evolved to cope with exogenous stresses. Likewise, the recent resolution of the structure of activated STATs has provided important insight into this signaling paradigm. With these tools it should be possible to develop a more detailed understanding of STAT-mediated signal transduction.

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