Transcriptional regulation by STAT1 and STAT2 in the interferon JAK-STAT pathway

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STAT1 and STAT2 proteins are key mediators of type I and type III interferon (IFN) signaling, and are essential components of the cellular antiviral response and adaptive immunity. They associate with IFN regulatory factor 9 (IRF9) to form a heterotrimeric transcription factor complex known as ISGF3. The regulation of IFN-stimulated gene (ISG) expression has served as a model of JAK-STAT signaling and mammalian transcriptional regulation, but to date has primarily been analyzed at the single gene level. While many aspects of ISGF3-mediated gene regulation are thought to be common features applicable to several ISGs, there are also many reports of distinct cases of non-canonical STAT1 or STAT2 signaling events and distinct patterns of co-regulators that contribute to gene-specific transcription. Recent genome-wide studies have begun to uncover a more complete profile of ISG regulation, moving toward a genome-wide understanding of general mechanisms that underlie gene-specific behaviors.

Introduction

The JAK-STAT pathways were first characterized in the type I interferon (IFN) response, which includes IFNβ and diverse IFNα subtypes.[1,2] Unique among JAK-STAT pathways, type I IFN stimulation of mammalian cells activates a heterotrimeric transcription factor that consists of a SH2-phosphotyrosine-mediated heterodimer of STAT1 and STAT2 in association with IFN regulatory factor 9 (IRF9). This STAT-containing complex binds to an IFN-stimulated response element (ISRE) in IFN-stimulated gene (ISG) promoters, and was named ISGF3. ISGF3 is the driver for type I IFN-stimulated transcriptional activation, which is an essential primary barrier for virus infection and important precursor for activation of subsequent innate and adaptive antiviral immune responses.

The importance of STAT1 and STAT2 in antiviral immunity is highlighted by the analysis of mice with targeted disruptions in individual STAT loci. STAT1-deficient mice were found to be highly sensitive to diverse viruses including vesicular stomatitis virus, influenza virus, and herpes simplex virus, in addition to

bacterial pathogens such as *Listeria monocytogenes*.[3-6] However, the STAT1-deficient mice respond normally to several non-IFN cytokines. STAT2-deficient mice also exhibit increased susceptibility to viral infection and enhanced replication of vesicular stomatitis virus and dengue virus, among others.[7,8] Loss of STAT2 results in reduced STAT1 steady-state levels and impaired STAT1 homodimer formation in addition to preventing the activation of ISGF3. Consequently, the STAT2 deficiency correlates with defective IFN autocrine/paracrine signaling, which creates additional tissue-specific immune defects related to the response to IFN stimulation.

STAT1 and STAT2 are key components of the transcription factor complex in the IFN signaling pathways. The sole type II IFN, IFNγ, represents the canonical JAK-STAT signaling paradigm, resulting in an SH2-phosphotyrosine-mediated homodimeric STAT1 transcription factor known as the gamma-IFN activated factor (GAF).[1] In addition to type I IFN, ISGF3 can be activated by type III IFNs, consisting of IFNλ1, IFNλ2, and IFNλ3.[9] Although type I and type III IFNs use distinct transmembrane receptors to initiate their signaling cascades, they converge upon ISGF3 as the active STAT transcription regulator. Nonetheless, despite the common ISGF3 factor, gene expression microarray analysis has demonstrated that IFNλ and IFNα do not result in identical gene expression profiles.[10] Stimulation with IFNλ was found to induce only a subset of the genes regulated by IFNα. Several well-known ISGs that are highly expressed in response to IFNα were minimally or not detected in the IFNλ microarray analysis, while other ISGs with relatively lower expression in response to IFNα were more readily detected in the IFNλ system. There are many potential explanations for this differential activity of ISGF3, including differences in IFN dosages or the presence of other factors that might divert ISGF3 to alternate loci. This single example of contextual diversity in STAT-mediated gene regulation suggests that while the STAT-containing transcription factors are key elements of biological responses, their activation alone may be insufficient to explain the complete extent of transcriptional regulation in any specific biological response system.

To better appreciate the extent and idiosyncrasies of gene regulation by ISGF3, contemporary whole-genome approaches and computational analysis will be needed to provide detailed information about transcription factor interactions with genomic loci in the context of specific stimuli. In fact, the current knowledge of transcriptional regulation by ISGF3 in the IFN systems is derived in large part from the generalization of conclusions that were based

on small-scale studies of relatively few representative target genes activated by type I IFN stimulation. Broader data sets representing more elaborate analysis of chromatin target sites are required to examine the regulation at the genome-wide level. This article reviews these generalized conclusions regarding the activity and co-regulators of ISGF3 in transcription regulation and explores the current status of genome-scale analysis of STAT1 and STAT2.

ISGF3 Assembly and Nuclear Translocation

Stimulation of cells with type I and type III IFN activates a typical JAK-STAT signaling program to form ISGF3 (**Fig. 1A**). JAK1 and TYK2 are activated by receptor engagement to phosphorylate the intracellular domains of the IFNα/β receptors or IFNλR1/ IL-10R2 receptors (for type I or type III IFN, respectively), which provide recruitment sites for the latent STAT1 or STAT2 SH2 domains.[9,11-16] At these sites, phosphorylation of STAT1 and STAT2 primarily drives dimerization and formation of the ISGF3 complex. The formation of the ISGF3 complex is thought to occur by sequential phosphorylation of STAT1 and STAT2.[17,18] It has been suggested that STAT2 is capable of pre-associating with the IFNAR2 chain, where it can become phosphorylated first, providing a docking site for the STAT1 SH2 domain. However, low levels of STAT1 phosphorylation by the IFNα/β receptor can generate the IFNα-activated STAT1 dimer, sometimes referred to as AAF, that is essentially the same factor as GAF (**Fig. 1B**).[18,19]

STAT1 and STAT2 interact with IRF9 to form ISGF3. Essential contacts between IRF9 and STAT1 are needed for ISGF3 stability and transcriptional activity.[20] IRF9 binds well to STAT2 in both unstimulated and IFN-stimulated cells and is likely to be pre-associated with any receptor-bound STAT2.[21-23] In the absence of STAT1 or STAT2, IRF9 is thought to be transcriptionally inert. However, a STAT2/IRF9 complex has been described to be sufficient to activate ISRE-driven transcription (**Fig. 1B**).

Both STAT1 and STAT2 shuttle between the nucleus and cytoplasm in unstimulated cells, but phosphotyrosine-mediated STAT dimers accumulate in the nucleus.[23,24] IRF9 contains a bipartite nuclear localization signal (NLS) and provides nuclear import capabilities to unphosphorylated STAT2, while STAT2 provides a strong nuclear export signal (NES) which primarily keeps the complex cytoplasmic.[22,23] Latent STAT1 shuttles in and out of the nucleus despite the lack of a typical NLS.[24] However, phosphotyrosine-mediated dimerization of STATs creates a strong NLS that allows STAT dimers to translocate into the nucleus with high efficiency.[24,25] While the exact mechanism of nuclear retention of tyrosine phosphorylated STATs is not completely understood, dephosphorylation is required for STAT1 nuclear export in the type II IFN pathway.[26,27] Since STAT2, like STAT1, is exported from the nucleus by the CRM1 exportin, it is possible that a similar dephosphorylation mechanism might regulate ISGF3 nuclear retention.[23]

ISGF3-DNA Interaction

A conserved DNA sequence element was recognized in type I ISG promoters and characterized as the IFN-stimulated response element (ISRE).[28,29] The ISRE consensus DNA sequence, 5'-AGTTTCNNTT TCNC/T-3', is found at the promoters of most direct type I/III ISGs.[1] ISGF3 binds the ISRE specifically through IRF9 recognition of the core sequence 5'-TTCNNTTT-3', STAT1 interaction with the TTT motif near the 3' end of the ISRE, and STAT2 generalized contacts with guanine and cytosine nucleotides.[1,29,30] STAT1 can also bind to the gamma interferon activation site (GAS) element, 5'-TTCN$_{2-4}$GAA-3', as a phosphotyrosine-mediated homodimer.[31] In most cases the direct interaction of ISGF3 with individual ISG promoters after IFN stimulation has not been demonstrated, but is inferred from the presence of a promoter-proximal ISRE and immediate IFN-induced mRNA expression. These features are likely to be diagnostic for ISGF3 responsive regulation, and is confirmed by more detailed studies of a few well-known ISGs such as ISG15 and ISG54 (also known as IFIT2).[29]

ChIP-chip analysis of STAT1 and STAT2 targets. With the advent of chromatin immunoprecipitation (ChIP) analysis, coupled to quantitative gene-specific PCR (ChIP-qPCR), screening of DNA microarrays or tiling arrays (ChIP-chip), or high-throughput DNA sequencing (ChIP-seq) methods, the interaction between ISGF3 components and specific DNA loci can be simultaneously examined genome-wide (**Table 1**). Analysis of IFNα- and IFNγ-activated STAT1 and STAT2 binding to human chromosome 22 using ChIP-chip confirmed four genes previously characterized as IFN-responsive, APOL1, APOL2, HIRA, and USP18, as direct STAT targets.[32] Many loci were identified to be bound by both STAT1 and STAT2 after IFNα stimulation that had not been previously characterized. Some of these loci represent annotated genes, including RUTBC3 (also known as SGSM3) and SAM50, while others were linked to unannotated loci. The presence of IFN-activated STAT1 and STAT2 at unannotated loci might reflect a role in regulating non-coding RNA genes or may represent experimental artifacts. This ChIP-chip analysis also identified many target genes that were occupied by either only STAT1 or only STAT2 following IFNα stimulation, suggesting that ISGF3 may not be the only STAT factor relevant to IFNα responses.[32]

STAT2 primarily participates as a component of ISGF3 and other factors containing STAT2 are poorly understood. STAT2 homodimers have only been confirmed to form in the absence of STAT1 and under distinct biochemical conditions.[33] With both STAT1 and IRF9 present in the cells used in this ChIP-chip analysis,[32] STAT2 would form ISGF3 in lieu of STAT2 homodimers. Since STAT2 is also thought to require contact with specific IRF9 and STAT1 residues to stably bind DNA,[33] the loci bound by only STAT2, but not STAT1, are unlikely to be bound by STAT2 homodimers and may be attributed to the STAT2/IRF9 complex described earlier.[21] ChIP analysis of IRF9 executed in parallel with STATs would be instrumental to characterize the STAT2/IRF9 complex more thoroughly.

Unique STAT1 targets on human chromosome 22 can be attributed, at least in part, to STAT1's ability to homodimerize in IFNα-stimulated cells and bind to GAS elements in gene promoters independent of STAT2.[19,31,34] The ability of STAT1 to bind to GAS elements, a feature of the type II IFN system, even
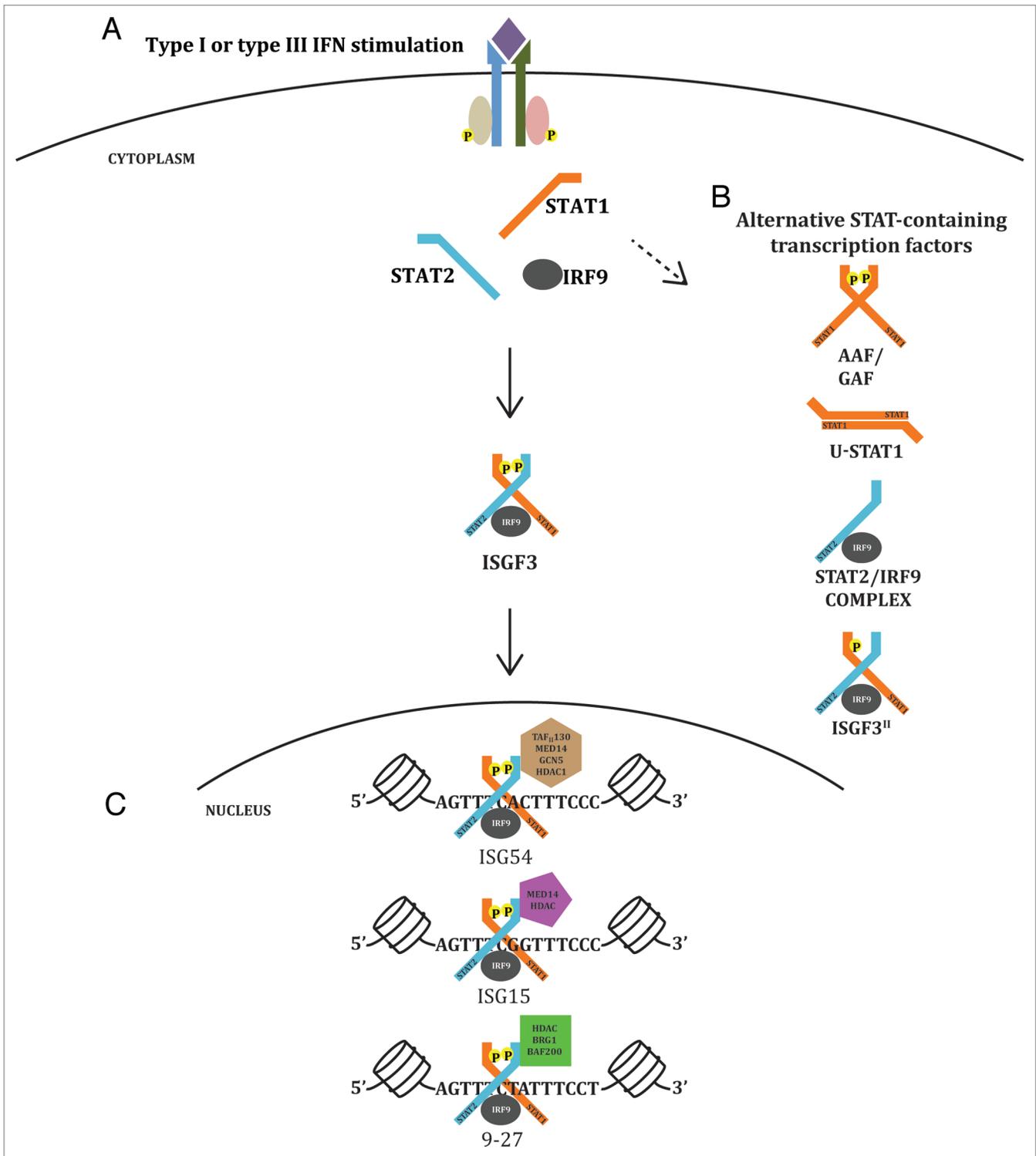
Figure 1. Diagrammatic representation of transcription regulation in response to IFN stimulation. (**A**) Binding of type I or type III IFN to their cognate receptors initiates a signaling cascade that results in phosphorylation and heterodimerization of STAT1 and STAT2 and association with IRF9 to form the active transcription factor complex ISGF3. (**B**) STAT1 and STAT2 primarily associate to form ISGF3, but have also been reported to form other transcription factor complexes in response to IFN stimulation. These include AAF/GAF, U-STAT1, a STAT2/IRF9 complex, and ISGF3^{II}. AAF/GAF has been widely reported to form in response to IFNα stimulation but the other complexes are less well understood. Accumulation of U-STAT1 has been shown to regulate ISG transcription; however, the structure and composition of the active transcription factor are not yet known. A STAT2/IRF9 complex has been reported to be transcriptionally active when overexpressed and ISGF3^{II} has been identified in a single cell line. (**C**) ISGF3, the canonical transcription factor, regulates the transcription of many ISGs. However, the co-regulators necessary for gene expression vary from gene to gene. Three ISGs, ISG54, 9–27, and ISG15, are depicted here along with their gene-specific co-regulators as examples of the differential regulation in the IFN pathway.

Table 1. Summary of (-/+) IFN-stimulated ChIP-chip and ChIP-seq data available

Factor	Condition	ChIP-chip	ChIP-seq
STAT1[32,41,42]	Untreated	+	+
	IFNα	+	+
	IFNγ	+	+
	IFNλ	–	–
STAT2[32,41,42]	Untreated	+	–
	IFNα	+	+
	IFNγ	–	–
	IFNλ	–	–
Pol II[41,42,53]	Untreated	+	+
	IFNα	–	+
	IFNγ	–	+
	IFNλ	–	–
IRF1[41,42,61,62]	Untreated	+	+
	IFNα	–	+
	IFNγ	+	+
	IFNλ	–	–
c-Myc[41,42,63]	Untreated	+	+
	IFNα	–	+
	IFNγ	–	+
	IFNλ	–	–
c-Jun[41,42,63]	Untreated	+	+
	IFNα	–	+
	IFNγ	–	+
	IFNλ	–	–

+, publicly available; -, not available.

under type I IFN stimulation allows STAT1 to participate in the regulation of both ISRE and GAS-containing loci independent of the type of IFN stimulus; this results in an overlapping pattern of target gene expression in the type I/III and type II IFN responses. Two genes, 9–27 (also known as IFITM1) and GBP, were among the first ISGs shown to respond to both IFNα and IFNγ.[35,36] 9–27 contains an ISRE and is expressed at high levels in both the type I/III and type II IFN systems, while GBP contains overlapping ISRE and GAS sequences. A microarray gene expression study also identified other ISGs including TAP1 (also known as RING4) and IRF1 to be inducible by both type I and type II IFNs.[37]

Genome-wide STAT1 and STAT2 occupancy. A ChIP-seq experiment assessed STAT1 occupancy genome-wide in both unstimulated and IFNγ-stimulated cells and examined 31 genes with known GAS or ISRE sites more closely.[38] Within the 31 genes, some genes, including TAP1 and IRF1, exhibited STAT1 binding at both steady-state and after IFNγ stimulation. In contrast, genes like GBP1 were only bound by STAT1 after IFNγ stimulation. It was also discovered that STAT1 binds to many known ISRE-containing sites, including 9–27, after IFNγ stimulation. Unexpectedly, STAT1 did not bind to some genes

containing characterized GAS elements, such as CD40 and SERPINA3. It is not clear why STAT1 binds to some GAS-containing loci and not others. Interestingly, the 5' end of GAS is reminiscent of the 3' end of ISRE prompting the potential for a non-ISGF3 STAT1 complex to also bind at ISRE sites; more detailed studies of these consensus sequences and their accessibility are needed to fully understand how STAT1 regulates transcription of its target genes.

DNA recognition by ISGF3 is also influenced by post-translational modifications to STAT1. A gene expression study evaluating the kinase, IKKε, revealed its absence affected a large number of ISGs differentially in response to type I IFN.[39,40] Some ISGs including ISG60 and ADAR1, exhibited decreased expression levels, while others, such as PRKRA, remained unaffected in the absence of IKKε. IKKε was also found to phosphorylate serine 708 of STAT1 and inhibit AAF/GAF formation in response to IFNβ. In the absence of IKKε, the ISRE of a few ISGs, OAS1b, MX1, and ADAR1, had decreased levels of ISGF3 binding. A STAT1α ChIP-seq analysis done to further understand the role IKKε plays in the type I IFN response revealed GAF-dependent targets were enhanced compared with ISGF3-dependent targets in the absence of IKKε.[39] This indicates IKKε affects type I ISG expression indirectly by influencing the binding ability of STAT1α at specific target genes. Predominantly GAF-dependent genes such as NOS2 were still bound by STAT1α, while some ISGF3-dependent genes had decreased STAT1α binding levels in the absence of IKKε. This genome-wide ChIP-seq experiment uncovered the extent of altered patterns of STAT1α binding under specific conditions at single gene and genome-wide levels.

The recent Encyclopedia of DNA Elements (ENCODE) project brings a more sophisticated level of STAT occupancy pattern analysis, with publically accessible data sets featuring both STAT1 and STAT2 ChIP-seq experiments (**Fig. 2 and Table 1**).[41,42] The currently available ENCODE data set provides a comprehensive and unbiased view of STAT1 and STAT2 genome occupancy following IFN stimulation, but corresponding steady-state data are not available.[41] Therefore, it is impossible to directly compare the steady-state and IFN-stimulated patterns of STAT1 and STAT2 occupancy without further experimentation. IFN-stimulated STAT1 ChIP-seq was performed in K562 cells, but untreated STAT1 ChIP-seq was done in GM12878 cells. Untreated STAT2 ChIP-seq is not publically available at this time, allowing no comparison with the IFN-stimulated STAT2 data from K562 cells. Nonetheless, indirect comparisons can be made for individual loci (**Fig. 2**).

Examination of sequence tag density signals of STAT1 and STAT2 will help to elucidate and compare binding patterns at various loci. Clear peaks of STAT1 and STAT2 occupancy after 30 min or 6 h of IFNα stimulation surround the promoter of ISG54, a well-known ISG, in K562 cells (**Fig. 2A**). Steady-state STAT1 ChIP-seq from GM12878 cells reveals no specific peak at the ISG54 promoter. The high, apparently inducible, STAT1 and STAT2 signal at the ISG54 promoter reaffirms it as a direct target of ISGF3. Similarly, putative ISGs can be reevaluated as targets of STAT1 or STAT2 (**Fig. 2B**). For example, RUTBC3, previously implicated by ChIP-chip analysis, only exhibits weak
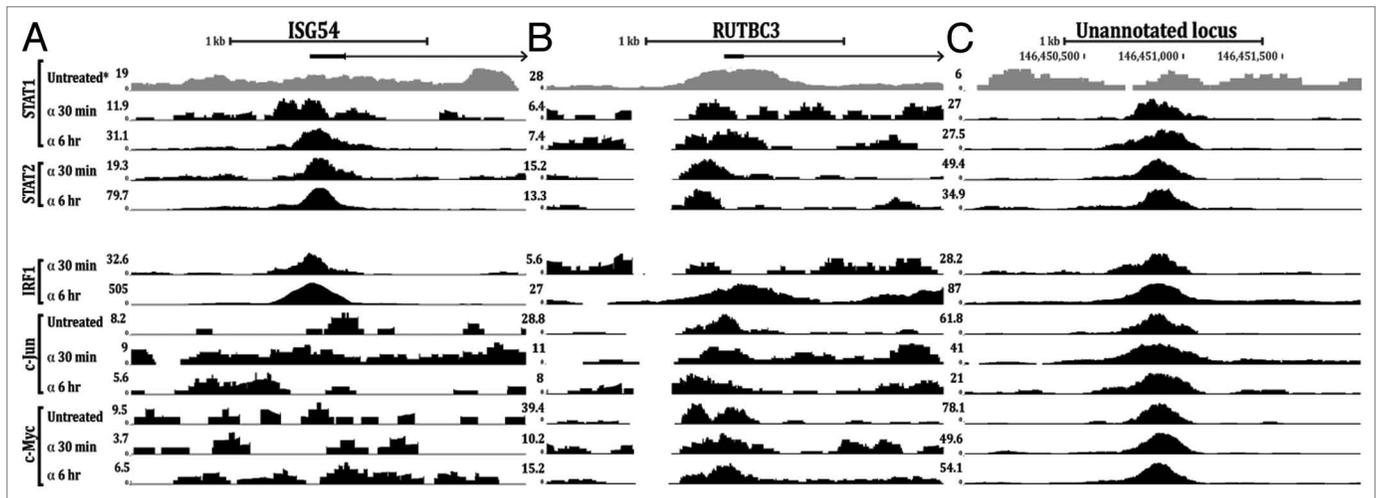
Figure 2. ChIP-seq profile at ISG54, RUTBC3 and an unannotated locus. Sequence tag density signals from ENCODE ChIP-seq data sets are shown for untreated, 30 min IFNα-stimulated or 6 h IFNα-stimulated K562 cells (black) and GM12878 cells (gray). STAT1 and STAT2 occupancy is shown in the top portion at ISG54, a classical ISG (**A**), RUTBC3, a gene upregulated by IFNα (**B**) and an unannotated locus on chromosome 2 at position 146,449,900–146,451,900 (**C**). Co-occupancy at these loci by IRF1, c-Myc and c-Jun, is shown in the bottom portion of the figure. The individual tracks were auto-scaled to allow visualization of the binding pattern, especially when the signal differs drastically between loci. *Indicates data was not generated from the same experiment as the IFNα-stimulated data.

STAT-specific signals even after 6 h IFNα stimulation, suggesting that it is not a direct STAT target gene.[32] These data sets also allow exploration of uncharted regions of the human genome by examination of STAT binding at new annotated genes, unannotated loci, and non-protein-coding regions. For example, a peak of STAT1 and STAT2 occupancy is observed at an unannotated region of chromosome 2 at position 146 449 900–146 451 900, and appears to be dependent on IFN stimulation (**Fig. 2C**). Further experimentation will be required to determine the significance of this phenomenon.

Non-Canonical STAT Transcription Factors

The conventional notion that IFN-activated transcription factors only consist of phosphorylated STATs is being challenged with growing evidence of unphosphorylated STATs playing a role in gene regulation. Gene expression analysis of STAT1-deficient cells that had been reconstituted with an unphosphorylatable Y701F STAT1 mutant revealed increased expression of some ISGs that were previously characterized as canonical ISGF3 targets, including the well-known OAS1 and IFI27.[43] Similar results were described in prolonged IFNβ treatment, which results in an increase in total STAT1 expression that is not tyrosine phosphorylated. Unphosphorylated STAT1 (U-STAT1) predominantly exists as a dimer,[44,45] but has been shown to act in a complex with IRF1 to induce LMP2 gene expression (**Fig. 1B**).[46] Further studies are needed to determine whether U-STAT1 acts alone or in a complex when regulating ISG expression.

The phenomenon of transcriptional regulation by unphosphorylated STATs has only been minimally investigated for STAT2. ChIP-chip analysis of 113 ISRE-containing gene promoters, such as MX1 and IFI6, found that while most ISGs are marked by phosphorylated STAT2 recruitment following IFNα

treatment, some genes were found to contain unphosphorylated STAT2, invoking the involvement of a transcription factor complex distinct from ISGF3.[47] However, this study was limited by the indirect method of identifying unphosphorylated STAT2 gene targets through a sequential ChIP with a pan-STAT2 antibody followed by a phospho-STAT2 specific antibody. Future experiments using STAT2 proteins that are unable to be phosphorylated, especially at times when unphosphorylated STATs are known to accumulate, might confirm these results. Unphosphorylated STAT2 may also associate with tyrosine-phosphorylated STAT1 and IRF9 to form ISGF3[II] and stimulate low levels of ISRE-containing gene expression in response to prolonged IFNγ treatment (**Fig. 1B**).[48] However, it remains to be determined whether this is a common transcription factor since this complex has only been reported in a single cell line. These studies do suggest that the simple canonical model of tyrosine-phosphorylated STAT1 and STAT2 interacting with IRF9 to form an active transcription factor may not completely explain the range of transcriptional regulation phenomena associated with STAT-driven target genes in the IFN response and points toward the need for additional genome-scale studies of STAT protein occupancy.

Transcriptional Co-Regulators

ISGF3 stimulates transcription of target genes by recruiting co-regulators that can affect DNA accessibility, interact with transcriptional machinery, modify histones, and influence RNA polymerase II (Pol II) elongation (**Fig. 1C**). Both STAT1 and STAT2 are known to interact with transcriptional co-activation machinery, but STAT2 has a prominent transcriptional activation domain at its C-terminus that has been recognized as the predominant ISGF3 component responsible for recruiting

Table 2. Examples of co-regulators required for ISG-specific expression

	ISG15	ISG54	9–27	IFI27	IFITM3	6–16
TAF$_{II}$	ND	TAF$_{II}$130[54]	ND	ND	ND	ND
Mediator	Med14[56]	Med14[56]	ND	ND	ND	ND
HAT	ND	GCN5[54]	ND	ND	ND	ND
HDAC[50-52]	+	+ (HDAC1)[50]	+	+	+	+
BRG1[58,59]	−	ND	+	+	+	−
BAF200[60]	ND	ND	+	+	−	ND

ND, not determined; +, required; -, not required.

co-regulators.[49] Functional analysis of ISGF3-co-regulator interactions have largely been examined at the single gene and reporter-gene transcription levels with the prominent exception of histone deacetylase (HDAC) activity.

Histone modifiers. Although deacetylation is commonly associated with transcriptional repression, HDAC activity was reported to be required for IFN-stimulated transcription and antiviral immunity.[50] HDAC activity was demonstrated to be required for the transcription of ISG54 and 9–27, and virtually all of the ISGs examined in a microarray gene expression analysis (**Fig. 1C and Table 2**).[50-52] STAT1 and STAT2 co-immunoprecipitated specifically with HDAC1 and not HDAC4 or HDAC5, but siRNA knockdown of HDAC1 only partially decreased ISG54 expression, suggesting the involvement of more than one HDAC protein in ISG regulation. The exact mechanism of how HDAC activity promotes ISG transcription is not known since STAT phosphorylation, ISGF3 assembly, nuclear translocation and DNA binding were not affected by HDAC inhibition. The absence of Pol II at the ISG54 promoter in a ChIP assay following HDAC inhibitor and IFNβ treatment suggests HDAC activity is necessary for Pol II recruitment to an ISG. However, Pol II recruitment to IRF1 was still intact despite the presence of HDAC inhibitors. As IRF1 is regulated by IFN-activated STAT1 homodimers, this indicates ISGF3 and GAF target genes have different requirements for HDAC activity. Whether HDAC activity is generally required for Pol II recruitment at many type I IFN target genes, as it is with ISG54, remains to be determined. Currently the status of Pol II occupancy before and after IFN induction can be explored through available ChIP-chip and ChIP-seq data (**Table 1**).[41,42,53] The generality of Pol II recruitment to ISG promoters vs. other activation schemes including release of paused RNA polymerase will be revealed by a more extensive evaluation of RNA polymerase localization and phosphorylation before and after IFN stimulation.

Interestingly, STAT2 also associates with histone acetyltransferases (HATs), which have an opposing function to HDACs. STAT2 residues 811–814 were identified to be necessary for HAT recruitment and were found to interact with the GCN5 protein in co-immunoprecipitation assays.[54] GCN5 was required for ISG54-luciferase reporter gene transcription, and recruitment of STAT2 to the ISG54 promoter in a ChIP assay coincides with histone H3 acetylation (**Table 2**). STAT2 also interacts with p300/CBP, HATs with ubiquitous interactions in the cell, but not via the necessary residues 811–814 for HAT activity, indicating these proteins may not be required for gene-specific expression patterns.[54,55]

Transcriptional machinery and mediator. In addition to HAT and HDAC activity, subunits of the RNA polymerase-associated transcriptional machinery and Mediator complex can also influence the type I IFN-induced transcriptional response and Pol II recruitment. Several subunits of the TFIID transcriptional machinery were tested for enhancement of ISG54-luciferase reporter activity (**Table 2**).[54] Surprisingly, the TATA binding protein (TBP) is not required for ISG54 expression and overexpression of TBP can instead inhibit ISG54-luciferase activity. One TATA-binding protein associated factor, TAF$_{II}$130, was found to enhance type I IFN transcriptional responses, while TAF$_{II}$28 and TAF$_{II}$250 did not. The Mediator subunit, MED14, enhances ISRE-luciferase driven transcription and interacts with STAT2 and the ISG54 promoter (**Table 2**).[56] In contrast, MED17, another Mediator subunit, associates with STAT2 but does not enhance transcription. However, the presence of the aforementioned TAF and MED proteins are limited to luciferase reporter and ChIP-qPCR assays and more broad studies are needed to determine the extent these co-factors can be generally found at ISG promoter regions in response to type I IFN.

Chromatin remodelers. These small- and large-scale studies suggest that a common set of transcriptional co-activators regulate the expression of diverse ISGs. The requirements for BRG1, a subunit of the SWI/SNF chromatin remodeling complex,[57] are apparently more gene-specific.[58,59] BRG1 was found to associate with STAT2 in response to IFNα, and its importance for the regulation of six ISGs was tested. Two of the ISGs tested, IFI27 and 9–27, were found to require BRG1 for IFNα-induced expression (**Table 2**). However, the ISGs 6–16, ISG15, STAT1, and STAT2 were not sensitive to BRG1. A similar study identified additional BRG1-dependent ISGs, including the well-studied IFITM3. Another subunit of the SWI/SNF complex, BAF200, was found to be required for expression of ISG 9–27, but not IFITM3.[60] The extent to which BRG1 or BAF200 differentially regulates additional ISGF3 targets remains to be determined genome-wide, but it is likely that additional co-factors exhibit similar gene-specific behavior in ISG transcriptional regulation (**Table 2**).

These small-scale studies have already identified a number of proteins that functionally interact with ISGF3 and demonstrate differential regulation of ISGF3 targets in a co-regulator-specific manner (**Fig. 1C**). Although the ENCODE ChIP-seq data primarily identifies protein-DNA interactions, co-occurrence by non-STAT1 or -STAT2 proteins at ISG promoter loci bound by STAT1 or STAT2 can provide a list of proteins that may play a role in the IFN response. The current ENCODE ChIP-seq data examined 119 transcription-related factors including chromatin remodellers and histone modifiers that can be explored.[42] A majority of the non-STAT factors were surveyed at steady-state, but a few were also tested after IFNα stimulation including IRF1, c-Myc and c-Jun (**Fig. 2 and Table 1**). A strong peak of IRF1, co-occurring with IFNα-stimulated STAT1 and STAT2 peaks, was found at ISG54 and weaker patterns

were observed at RUTBC3 and the unannotated locus on chromosome 2 in IFNα-treated cells. Binding of c-Jun and c-Myc did not exhibit a defined peak at ISG54 and RUTBC3; however, both c-Jun and c-Myc exhibited clear co-occupancy with STAT1 and STAT2 at the unannotated locus. Similar analysis can be used to generate hypotheses for further study, but whether these factors play an active role in type I IFN regulation at these genes will require more precise experimentation.

Perspectives

The IFN-JAK-STAT pathway is a primary innate antiviral system driven by gene regulatory networks, but was established by detailed and insightful investigation of a small subset of ISGs. Contemporary studies involving large-scale, genome-wide methods are capable of revealing more complex transcriptional regulatory phenomena mediated by STAT1 and STAT2 at all target sites. The use of quantitative assays such as ChIP-seq, along with nucleosome profiling and proteomic approaches, will help build on the current understanding of differential regulation of target genes. Additionally, as technology continues to evolve and methods based on chromatin conformation capture become more utilized, we may be able to examine beyond the local DNA-protein and protein-protein interactions and discover STAT interactions at distal regions. Insights at the genomic level will enable us to continue deciphering the many mechanisms of STAT1- and STAT2-regulated transcription.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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