

Mechanisms of Type I Interferon Cell Signaling and STAT-Mediated Transcriptional Responses

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Abstract

The interferons are pleiotropic cytokines that are induced in response to virus infection and act in a paracrine fashion to elicit an antiviral state in nearby cells. Binding of interferons to their cell surface receptors induces a tyrosine kinase signaling cascade that leads to the activation of latent cytoplasmic signal-transducer-and-activator-of-transcription (STAT) factors. Activated STATs then translocate into the nucleus and are targeted to conserved promoter-enhancer sites to induce the transcription of interferon-responsive genes that encode for proteins with potent antiviral, growth-inhibitory, antitumor, and immunomodulatory properties. Although the signaling and activation phase of the interferon response has been well characterized, several recent findings have further clarified the cellular events that immediately follow STAT activation, including the identification of the amino acid signals that regulate the subcellular distribution of interferon-signaling proteins. To achieve their full transcriptional capacity, members of the STAT family of transcription factors have been shown to require interactions with an assortment of nuclear transcriptional co-activator proteins. A number of the STAT co-activator protein partners have only been identified recently. Some of these interactions suggest cross-talk with other signaling pathways, thereby reaffirming the far-reaching, yet undiscovered, properties of interferons.

Key Words: Interferon, transcription, co-activators, chromatin, STAT1, STAT2, p48, IRF9, nuclear localization signals (NLS), nuclear export sequences (NES).

Glossary

ATP: adenosine triphosphate
BRCA: breast cancer susceptibility marker
cAMP: cyclic adenosine monophosphate
CBP: CREB-binding protein
CREB: cAMP-response-element-binding protein
Crm1: chromosome region maintenance 1
DNA: deoxyribonucleic acid
GAF: gamma-activated factor
GAS: gamma-interferon-activated sites
GFP: green fluorescent protein
GTF: general transcription factor
HAT: histone acetyltransferase
HDAC: histone deacetylase
IFN: interferon
IFNAR: IFN / receptor
IL-2: interleukin-2
IRF: interferon regulatory factor
ISG: interferon-stimulated gene
ISGF3: interferon-stimulated gene factor 3
ISRE: interferon-stimulated response element

JAK: Janus kinase
MCM: minichromosome maintenance
MHC: major histocompatibility complex
NES: nuclear export sequences
NLS: nuclear localization signal
NMI: N-Myc interactor
PIAS1: protein inhibitor of activated STAT1
PMSP: proline-methionine-serine-proline
PRMT1: protein arginine methyl-transferase
PSP: proline-serine-proline
RNA: ribonucleic acid
SH2: src homology 2
STAT: signal transducer and activator of transcription
TAD: transcriptional activation domain
TAF: TBP-associated factor
TBP: TATA-binding protein

Introduction

MOST CELLS POSSESS THE INNATE ABILITY to fight viruses. Virus infection directly activates the transcription of Type I interferon (IFN /) genes in infected cells. The secreted Type I IFN cytokines bind to their cell surface receptors on adjacent cells to activate the formation of the interferon-stimulated gene factor 3 (ISGF3) transcription factor complex, the primary regulator of antiviral gene transcription by IFN . ISGF3 is composed of two signal-transducer-

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and-activator-of-transcription (STAT) proteins, STAT1 and STAT2, and the interferon regulatory factor (IRF) protein IRF9 (also known as p48 or ISGF3 γ) (Fig. 1). The activated trimeric complex then becomes competent for translocation from the cytoplasm to the nucleus, to initiate the transcription of interferon-stimulated genes (ISGs) that encode for proteins with potent antiviral, antiproliferative, antitumor, and anti-inflammatory effects (1).

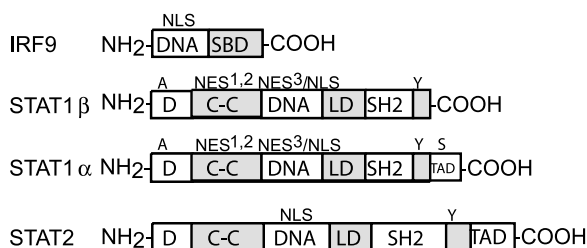


Fig. 1. Proteins of the ISGF3 complex. STAT1, STAT2 and IRF9 combine to form the Type I IFN-activated ISGF3 complex that mediates interferon-stimulated response element (ISRE)-dependent antiviral gene transcription. IRF9 is a 393-amino acid protein with an N-terminal DNA-binding domain (DNA) and a C-terminal STAT-binding domain (SBD). STAT1 β is an alternatively-spliced isoform of the 750-amino acid STAT1 α , which has the additional C-terminal 38-amino acid transcriptional activation domain (TAD). STAT1 is phosphorylated on Tyr701 (Y) and Ser727 (S). The 851-amino acid STAT2 is phosphorylated on Tyr689 (Y), but lacks a phosphoserine site in its TAD. The nuclear localization signals (NLS) are located within the protein domains as indicated. The three STAT1 nuclear export signals (NES¹⁻³) are located in the domains as shown. Post-translational modification sites: A=STAT1 PRMT1 arginine 31 methylation site; Y=tyrosine 701 phosphorylation site; and S=serine 727 phosphorylation site. D=domain responsible for dimer-dimer interactions, C-C=coiled-coil domain, DNA=DNA-binding domain, LD=linker domain, SH2=src-homology 2 domain, and TAD=transcriptional activation domain.

Activated T lymphocytes and natural killer cells also help regulate antiviral responses in the adaptive immune response by producing the Type II interferon (IFN γ) cytokines. IFN γ , which is distinct from but functionally related to Type I IFNs, binds to a different cell surface receptor, leading to the activation and formation of a homodimeric STAT1 complex, called the gamma-activated factor (GAF) complex. In addition to antiviral responses, IFN γ is also a key regulator of cell proliferation and is involved in tumor surveillance (2).

Although the activation of the ISGF3 and GAF transcription factor complexes has been well

studied in the last decade, since the discovery of STATs, comparatively little is known about how activated STAT proteins enter the nucleus, communicate with the ribonucleic acid (RNA) polymerase holoenzyme complexes to initiate gene transcription, and re-enter the cytoplasm once the transient transcriptional response to IFN has subsided. It has become apparent that components of the ISGF3 and GAF complexes are actively shuttled between the cellular compartments. Macromolecular transport across the nuclear pore complex in both directions is an energy-requiring, tightly regulated event for cargo proteins exceeding ~40-kDa (3, 4). A family of soluble shuttling transport receptors called importins or karyopherins mediates the nuclear import of proteins. During the nuclear import process, one of several proteins from the importin- α family recognizes and binds to its specific amino acid sequences called nuclear localization signals (NLS) on the cargo protein. Transport of proteins in the opposite direction, from the nucleus to the cytoplasm, is regulated by the family of exportin proteins that recognize characteristic leucine-rich nuclear export sequences (NES) on cargo proteins destined to leave the nucleus (5–8). Recent advances from several laboratories, including ours, have demonstrated that the subcellular distribution of ISGF3 protein components is regulated by novel NLSs and NESs (9–13).

Ample recent evidence (14) also demonstrates that the STATs, like many transcription factors, initiate gene transcription with the help of transcriptional co-activators. Transcriptional co-activators describe a broad range of nuclear proteins that are important in bringing about the full gene expression capacity of transcription factors. Co-activators have diverse functions, and those that have already been well characterized assist primarily in the delivery of activating signals to the basal transcription machinery that is present in most active genes, and/or in the remodeling of chromatin structure to allow transcription factor access to the promoter. The precise roles that the various STAT-co-activator protein interactions play in Type I and Type II interferon antiviral signaling pathways, and thus, in antiviral gene transcription, are still unclear and are the focus of intense research. This review will focus on the latest findings in IFN signaling following receptor activation, and examine several STAT-co-activator protein interactions and their potential importance in Type I (ISGF3-mediated) and Type II (GAF-mediated) IFN-dependent gene expression.

The Type I IFN Signaling Pathway

The Type I interferons are a gene family consisting of a single IFN β gene and at least twelve immediate-early and delayed-early IFN α genes that function in most cells by activating interferon-responsive gene expression (15–18). IFN α/β also regulates adaptive antiviral immune responses involving dendritic and natural killer cells (19). Unlike other STAT-signaling pathways, the Type I IFN pathway involves two protein families, the STATs and IRFs, which work together to form the ISGF3 complex that activates ISG expression (Fig. 2). Proteins encoded by ISGs have antiviral properties, and elicit their effects by interfering with cellular or viral processes such that viral replication becomes blocked or impaired (1). The direct inhibition of the viral replicative life cycle, arrest of cell cycle progression, and increasing of cellular susceptibility to apoptosis are common mechanisms by which antiviral proteins limit the extent of viral spread (20, 21). While a number of ISGs and IFN-induced antiviral proteins have been well defined (17), the biological functions for many remain unclear. A recent oligonucleotide microarray analysis has identified at least 300 ISGs induced by both IFN α and IFN β , many of which were previously unknown (22).

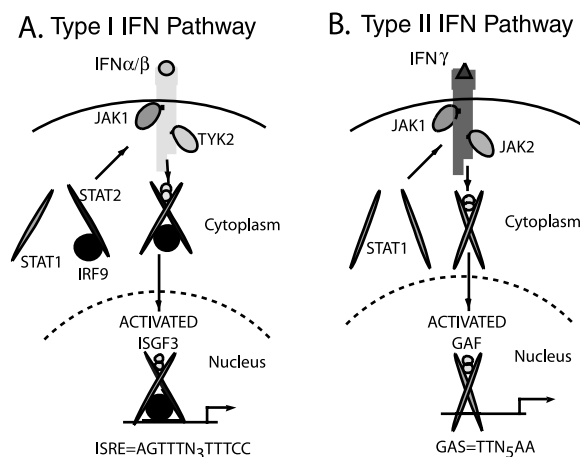


Fig. 2. The Type I and Type II IFN pathways. IFN α/β and IFN γ , which are functionally related but structurally distinct cytokines, bind to different cell surface receptors. IFN α/β activates the Type I IFN pathway, leading to the oligomerization of STAT1, STAT2, and IRF9 to form ISGF3. IFN γ activates the Type II IFN pathway, which directs the homodimerization of two STAT1 molecules to form GAF. Both activated ISGF3 and GAF complexes translocate into the nucleus where they bind to promoters with ISRE and GAS sequences, respectively.

Once the activated ISGF3 complex has entered the nucleus, the IRF9 component acts as a sequence-specific deoxyribonucleic acid (DNA)-binding protein and confers recognition upon the activated STAT1:2 heterodimer (23–26). The contribution of IRF9 as an adapter for the STAT1:2 heterodimer represents a unique feature of the ISGF3 complex, as other STAT-signaling pathways involve STAT homodimers that bind directly to DNA. The ISGF3 complex binds to and activates transcription of genes through the conserved interferon-stimulated response elements (ISRE; AGTTTN3TTTCC) (27).

STAT1 and STAT2 represent two of seven mammalian STAT family members that range in size from 90–115-kDa (28). Protein sequence alignment and crystallographic analyses of STAT1, STAT3, and STAT4 reveal that the STAT transcription factors collectively feature high amino acid homology over several distinct protein regions having defined structural and functional roles (29–31). From studies of the STAT4 crystal structure, the amino-terminal domain (residues 1–123) was found to stabilize reciprocal interactions between two STAT dimer complexes bound to tandemly arranged promoter sites (31). The DNA-binding cores of STAT1 and STAT3 have been crystallized and observations reveal several distinct structural domains: an α -helical coiled-coil region (residues 136–317 of STAT1), a highly-conserved DNA-binding domain which possesses an immunoglobulin-type fold (residues 318–488), a conserved src-homology 2 (SH2) domain (residues 577–683) that interacts with phosphorylated tyrosine motifs, and an α -helical linker domain (residues 488–576) that bridges the DNA-binding and SH2 domains (29). The carboxyl-terminal end of STATs was too unstable to resolve for crystallographic analyses, but the region has been determined to be the important transcription activation domain (TAD), a region that interacts with additional proteins (14). It is the NH₂-terminal coiled-coil domains of STAT1 and STAT2 that interact with IRF9 to form ISGF3 (19). The 48-kDa IRF9 protein possesses a conserved amino-terminal DNA-binding domain and a carboxyl-terminal STAT-binding domain (Fig. 1). With the exception of a nuclear localization signal in the DNA-binding domain (described below), additional functional domains within the IRF9 protein have not yet been described.

The Type II IFN Signaling Pathway

The Type II interferon, IFN λ , is encoded by a single gene, and plays an essential role in the regulation of the adaptive immune response. Binding of IFN λ to its receptor leads to the homodimerization of STAT1 proteins via intermolecular SH2-phosphotyrosine interactions to form GAF (Fig. 2) (32). The GAF complex then translocates into the nucleus to bind to conserved gamma-interferon activated sites (GAS; TTN₅AA) on the promoters of IFN λ -responsive genes. Unlike the ISGF3 complex, which uses the IRF9 protein as its DNA-binding adapter, the GAF complex binds to DNA directly. Many target genes induced by the IFN λ -activated GAF complex have been identified; further information can be found in references (2, 33).

STAT1 and STAT2 Are Essential for Innate Immunity

Mice deficient in STAT1 and STAT2 have been generated by gene targeting, and the observed phenotypes for both strains have revealed their important roles in the regulation of immune responses (34–36). STAT1 knockout mice are viable and undergo normal development. Based on studies from cell lines, a loss of STAT1 should affect both Type I and II IFN functions. Indeed, IFN-dependent responses were found to be absent, which increased their susceptibility to viral infections and certain microbial pathogens (34, 35). Numerous studies have also demonstrated the growth inhibitory properties of IFNs, suggesting that these cytokines play a role in controlling tumor growth. In support of this hypothesis, STAT1-deficient mice had a higher incidence of both spontaneous and methylcholanthrene-induced tumors than normal controls, suggesting a role for STAT1 as a tumor suppressor (37). Tumors also formed at an even higher rate in mice that were bred into a p53-null background. Together, these observations suggested the involvement of IFN λ in tumor surveillance. It is believed that a loss of STAT1 eliminates the ability for IFN λ to promote STAT1-dependent antigen processing and presentation by major histocompatibility complex (MHC) class I and II molecules, therefore allowing cancer cells to grow unchecked.

Like their STAT1 knockout mice counterparts, the STAT2-deficient mice are also viable and undergo normal development. As antici-

pated, STAT2 knockout mice were highly susceptible to viral infections due to defective IFN signaling (36). However, more subtle changes in fibroblasts derived from STAT2-deficient mice were identified. One interesting observation was that the levels of the STAT1 protein were lower in STAT2^{-/-} fibroblasts than in wild-type cells. This finding suggests that there may be a STAT2-dependent autocrine loop that maintains basal Type I IFN production and activity, which in turn sustains the steady-state expression of the interferon-responsive STAT1 gene. The loss of STAT2 therefore affected both Type I and Type II IFN-dependent transcriptional responses. The study also shows that macrophages, dendritic cells, and stromal fibroblasts derived from STAT2^{-/-} animals can no longer perpetuate more Type I interferons to support the autocrine/paracrine loops, thus altering their ability to regulate lymphocyte maturation and function (36). Future studies may reveal additional immunologic defects in STAT2-deficient mice.

ISGF3 Complex Assembly

While the mechanisms of Janus kinase-STAT (JAK-STAT) activation at the IFN λ receptor have been extensively studied, more recent work described in this section has begun to reveal the dynamics of trimeric ISGF3 complex assembly following IFN stimulation. Also, several reports have shed light on the mechanisms and amino acid signals that govern the subcellular distribution of the ISGF3 signaling components (9–13).

Binding of IFN λ to its cell surface receptor leads to the rapid receptor-dependent activation of latent cytoplasmic STAT1 and STAT2. Together with IRF9, they form the trimeric ISGF3 protein complex (38–40). Two subunit chains form the IFN λ receptor (IFNAR), the β chain or IFNAR1, and the α chain or IFNAR2, which is composed of the short (α_s) and long (α_L) transmembrane forms (Fig. 3) (41). Ligand binding induces dimeric aggregation of receptor chains, and activates receptor-associated Janus kinases JAK1 and TYK2. TYK2 then phosphorylates a specific tyrosine residue at position 466 on the cytoplasmic receptor tail of IFNAR1 (42). This phosphotyrosine serves as a docking site for the rapid recruitment of latent cytoplasmic STAT2 via its SH2 domain (43). Association of STAT2 with the cytoplasmic tail of the IFNAR α_L chain has

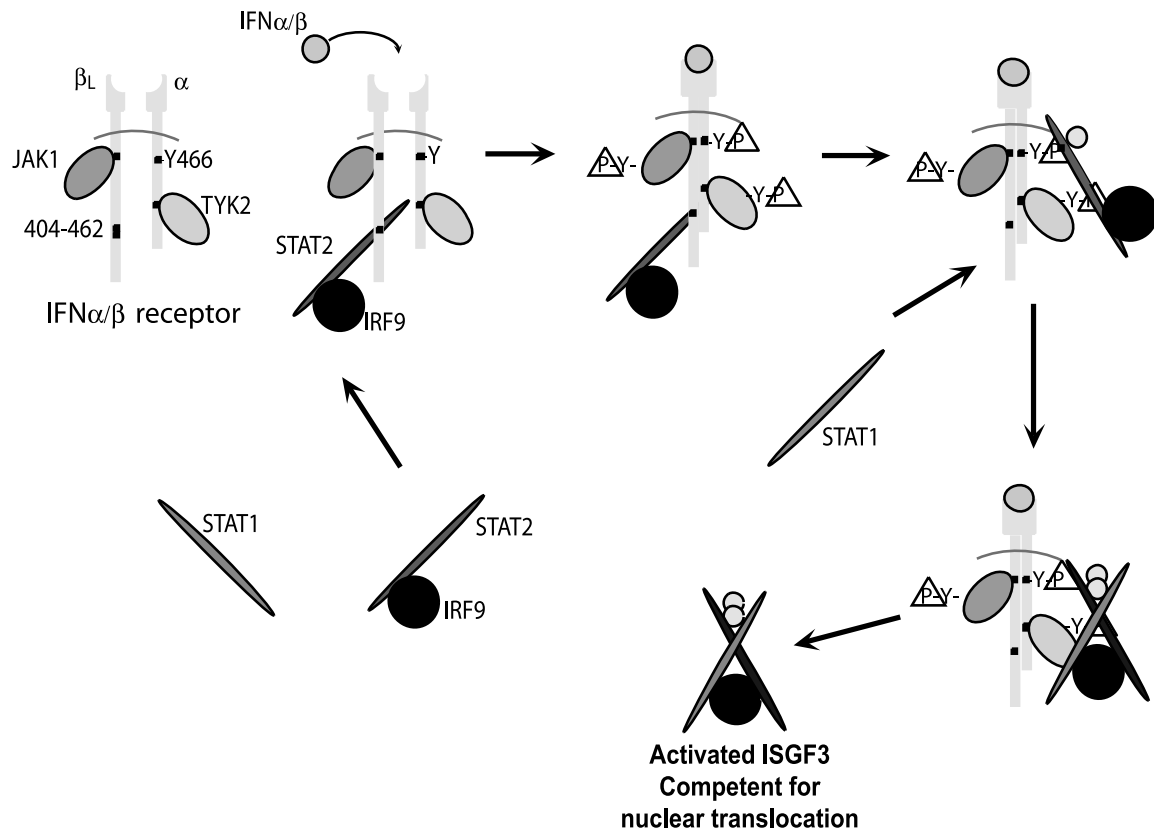


Fig. 3. The IFN α/β receptor and ISGF3 complex assembly. The IFN α/β receptor (IFNAR) is composed of two subunits—the IFNAR1 (α) and the IFNAR2 (β_L or β_S) chains. Constitutive and IFN activation-dependent STAT2-binding sites have been mapped at the cytoplasmic tail of IFNAR, as indicated. Binding of IFN α/β dimerizes the receptor and activates receptor-associated Janus kinases (JAKs) JAK1 and TYK2. TYK2 then phosphorylates tyrosine 466 on the α chain, which recruits STAT2 via its SH2 domain. Tyrosine 690 on STAT2 then becomes phosphorylated, which allows for the docking of the STAT1 protein. STAT1 also becomes phosphorylated at tyrosine 701, allowing for the heterodimerization of STAT1 and STAT2. The STAT1:STAT2 heterodimer is now competent for nuclear translocation. The IRF9 protein is associated with STAT2 in the cytoplasm and may play a role in ISGF3 complex assembly at IFNAR.

been reported to occur in both the absence (44) and presence (43) of Type I IFN signaling. Interestingly, the mapped regions for interaction with STAT2 within the IFNAR β_L cytoplasmic tail differ between the activated and inactivated states (45).

Once anchored to the receptor tail, STAT2 is then phosphorylated by TYK2 on tyrosine 690, which serves to recruit cytoplasmic STAT1 (46, 47). STAT1 is then subsequently phosphorylated on tyrosine 701, allowing for the heterodimerization of STAT1 and STAT2 (48). The activated STAT1:2 heterodimer forms while bound to the intracellular domain of the IFN receptor, and upon release, the complex becomes competent for nuclear import. It has generally been assumed that the STAT1:STAT2 heterodimer joins with their obligatory partner, IRF9, to form ISGF3 at some point during the course of translocation

into the nucleus, but the subcellular compartment where this event takes place was only recently defined. The IRF9 protein apparently plays an active role in ISGF3 complex assembly in the cytoplasm. Although the IRF9 protein was found to be a constitutively nuclear protein when expressed in cells (see below), the co-expression of the STAT2 protein retains IRF9 protein in the cytoplasm via direct protein interactions, indicating that the two proteins form cytoplasmic complexes even in the inactivated state (9). Interestingly, the cytoplasmic tail of the IFN α/β receptor β_L chain can recruit IRF9 only in the presence of STAT2 (Lau JF and Horvath CM, unpublished observations).

These observations suggest that the STAT2:IRF9 protein complexes could be involved with ISGF3 complex oligomerization at or near the receptor in the cytoplasmic com-

partment. The presence of STAT2:IRF9 cytoplasmic complexes at the IFN receptor may also provide a ready protein pool for rapid ISGF3 complex assembly, which is characteristic of the fast activation kinetics observed in the interferon response.

ISGF3 Subcellular Distribution

The subcellular distribution of STAT proteins is regulated in response to extracellular stimuli. While localized within the cytoplasm during the inactivated state, STAT complexes can be found within the nucleus after several minutes of IFN treatment (23). In the Type II IFN pathway, STAT1 proteins are recycled and returned to the cytoplasm within hours of their inactivation by a yet-to-be-identified nuclear phosphatase (49, 50). Further, the STAT2 protein exhibits a similar activation-inactivation cycle following IFN treatment, suggesting that the recycling of STAT2 to the cytoplasm is an essential component of Type I IFN signaling (Radulescu VC and Horvath CM, unpublished observations). The STAT proteins also move about within the cell in a haphazard, “random-walk” fashion, even after activation by the JAK-receptor complex (51). Although shown to be important for certain transcriptional regulators such as the SMAD (similar to mothers against decapentaplegic) proteins of the TGF β signaling pathway, the translocation of activated STAT1 dimers from the cytoplasmic face of the cell membrane into the nucleus does not rely on movement along actin cytoskeletal or microtubular networks (51, 52).

Early efforts to determine the amino acid sequences that govern STAT transport through the nuclear pore were hampered by the lack of classical NLS or SV40-like NES motifs. An initial study demonstrated the importance of the highly conserved amino-terminal domain (residues ~1–134) in regulating the nuclear accumulation of STAT1 (53). Also, STAT1 has been demonstrated to interact with the importin/karyopherin protein NPI-1, therefore implying that STATs utilize importin/karyopherin proteins to gain access into the nucleus (54). Recently, several laboratories determined the chemical structures of the STAT NLS and NES. Determination of the surface structure of the STAT1 and STAT2 nuclear localization signals (NLSs) was facilitated by the three-dimensional crystal structure of STAT1 which identified an arginine/lysine-rich region in the DNA-binding domains of both STAT1 and STAT2 (10). Be-

cause an NLS is often characterized by the presence of basic amino acids (55), a surface region bearing high concentrations of arginine and lysine served as an attractive focus to identify NLS candidates. Mutation to these basic amino acids abrogated the ability of the modified STAT1 and STAT2 proteins to enter the nucleus, and therefore implicated the arginine/lysine-rich region in mediating contact with proteins involved in the nuclear import process. Further, while the STAT NLS was being determined, three different groups reported the identification of three different NESs within the STAT1 protein that regulated its nuclear export (11–13). Mutation to each NES site eliminated the ability for these modified STAT1 molecules to exit the nucleus in an exportin 1/chromosome region maintenance 1 (Crm1)-dependent process. Two NES sites lie within the coiled-coil region (12, 13), while the third resides within the adjacent DNA-binding domain (11). These sites may be situated closely to each other in the natively folded protein and therefore represent multiple contact points for the exportin machinery. It is still unclear whether the nuclear export of STAT2 and other STAT proteins is regulated similarly to STAT1, but these amino acids are conserved.

The remaining component of the ISGF3 complex, the IRF9 protein, was initially found in both the nucleus and the cytoplasm, regardless of IFN stimulation (23, 56). Since the apparent molecular weight of the IRF9 protein approaches the theoretical diffusion limit for the nuclear pore complex (which is approximately 50-kDa), IRF9 was hypothesized to diffuse freely between the cytoplasm and nucleus. The finding that a green fluorescent protein (GFP)-IRF9 fusion protein, that was well beyond the theoretical diffusion limit for the nuclear pore complex, is constitutively targeted to and accumulates within the nucleus when expressed in cells detracts from the diffusion theory. Using fragments of IRF9 fused to GFP, the nuclear import signals were mapped to two interdependent clusters of arginines and lysines located in its DNA-binding domain (9). The two basic amino acid clusters, spaced apart by ten amino acids, is in agreement with a variation of the bipartite NLS motif (57). The novel IRF9-bipartite NLS motif is also conserved in two closely related IRF proteins, IRF4 and ICSBP/IRF8, signifying the generality of this novel NLS in a subset of IRF proteins.

Thus, these findings suggest there may be two pools of IRF9—one that is constitutively

nuclear by virtue of its bipartite NLS, and a second that is complexed with STAT2 in the cytoplasm. The IRF9 protein, once regarded as an inert DNA-binding adapter for STATs, now appears to play an active role in regulation of ISGF3-cell-signaling events. One can envision that upon receptor activation by IFN γ , STAT2 may bring along IRF9 to the receptor for oligomerization with STAT1. Alternatively, an activated STAT1:STAT2 heterodimer may translocate into the nucleus to join IRF9 at the ISRE-containing promoter. Because IRF9 is actively transported into the nucleus, it will be important to determine whether the protein possesses transcriptional functions independent of STAT1 and STAT2.

ISGF3-Independent Functions of IRF9

Several reports suggest that the IRF9 protein indirectly modulates the activities of several diverse genes, including the IP-10 chemokine gene that is involved in inflammatory and neoplastic responses (58), and the enhancer-1 region responsible for regulating gene expression in the hepatitis B virus (59, 60). In a more recent study, the overexpression of IRF9 in a specific breast cancer cell line led to its resistance to treatment with antimicrotubule agents such as paclitaxel (61). Overexpression of STAT1 or STAT2, or treatment with IFN γ , did not produce the same drug-resistance phenotype, making this event independent of ISGF3 functions and IFN γ activation. Although the overexpression of IRF9 led to the expression of several interferon-responsive genes, it remains unclear whether IRF9 or any of the induced ISG proteins play a direct role in conferring drug resistance. Importantly, the study also identified that IRF9 was highly expressed in a number of breast and uterine tumor samples, suggesting that high IRF9 protein levels may serve as potential markers for drug resistance in specific tumor types.

Transcriptional Activation and ISGF3

While the ISGF3-independent transcriptional functions of the IRF9 protein are not entirely understood, the roles played by STAT proteins are better defined. The STAT proteins have carboxyl-terminal transcriptional activation domains that contribute to gene transcription. Because either the transcriptionally active STAT1 (the 91-kDa full-length protein), or its splice variant STAT1 Δ (lacking the C-terminal

38 amino acid TAD), can be used interchangeably for Type I IFN signaling, it has been generally accepted that ISGF3-mediated transcriptional signals derive from the STAT2 protein (Fig. 1) (46, 62). It has also been demonstrated previously that at least the C-terminal 40 residues from STAT2 are required for full ISG transcription (47). A protein fragment of at least 116 residues from the STAT2 carboxyl-terminal TAD was also shown in heterologous DNA-binding domain fusion experiments to contain the necessary determinants to make this domain a potent transcriptional activator in isolation (47, 63–66). Interestingly, there is little amino acid sequence homology between the human and murine STAT2 proteins at their carboxyl-termini, but both domains have been shown to interact with a distinct but overlapping set of cellular proteins, including the histone acetyltransferase cAMP-response-element-binding protein (CREB)-binding protein (CBP)/p300 (63, 64). Thus, despite divergent amino acid sequences between species, the functions of the STAT2 C-terminal TADs appear to be conserved.

The mechanisms by which the STAT2 TAD elicits ISG transcription remain obscure. However, the TADs of other STAT proteins employ a number of mechanisms to regulate transcription. One level of regulation is achieved through a cytokine-induced phosphorylation event that takes place within the STAT TAD. Several STAT proteins possess in their TADs a key serine residue within a conserved proline-methionine-serine-proline (PMSP, in STATs 1, 3, and 4) or proline-serine-proline (PSP, in STATs 5a and 5b) motif that becomes phosphorylated in response to cytokine treatment (67). Serine phosphorylation in both STAT1 and STAT3 is required for maximal transcriptional activities. When STAT1 lacks this serine residue (serine 727), only 20% of the wild-type transcriptional capacity is retained, producing defects in IFN responses (68). The STAT2 TAD does not possess the PMSP/PSP motif, and is therefore not regulated by serine phosphorylation. However, because the carboxyl-terminus of STAT2 TAD is indispensable for Type I IFN responses, it will be interesting to determine whether this region is post-translationally regulated in some other fashion.

Although it is becoming well documented that the carboxyl-terminal STAT TADs, and in particular, those of STAT1 and STAT2, are potent and specific co-activator recruiters, it is worthy to note that other STAT structural domains have been implicated in transcriptional

regulation. For instance, a novel functional interaction was found in a yeast two-hybrid screen between the coiled-coil domains of all STAT proteins (with the exception of STAT2) with a protein called Nmi (N-Myc interactor) (69). The Nmi protein enhanced STAT1-dependent transcriptional responses to both IFN and interleukin-2 (IL-2). In a separate study, a key pair of lysine and glutamic acid residues within the STAT1 linker domain was found to be important for Type I IFN GAS-dependent transcriptional activities (70), but the mechanism for this defect is unknown.

Much of the earlier work on STAT-protein post-translational regulation has focused on tyrosine and serine phosphorylation events. More recently, the activity of the STAT1 protein has been shown to be regulated by methylation as well. The protein arginine methyl-transferase (PRMT1) was found to associate with STAT1, which allowed for the methylation of a highly conserved arginine residue (arginine 31) located at the STAT1 amino-terminus (71). Interestingly, PRMT1 was previously reported to associate with the cytoplasmic tail of the IFN γ receptor in a yeast two-hybrid assay, and the elimination of PRMT1 activity by antisense oligonucleotides reduced Type I IFNs antiviral signaling capabilities (72). The use of a specific inhibitor to eliminate PRMT1 activity also impaired Type I IFN-dependent transcriptional responses, suggesting the importance of methylation in transcriptional responses involving STAT1. Thus, the post-translational modification of STAT1 appears to be as important as the integrity of the STAT2 carboxyl-terminus TAD in Type I IFN signaling. The methylation of STAT1 is thought to enhance its ability to bind DNA, and prevents its interaction with the protein inhibitor of activated STAT1 (PIAS1) protein, a known inhibitor of STAT1-dependent transcription.

Overview of Transcriptional Activation

Gene transcription has been shown to be a dynamic process involving numerous protein factors and DNA-chromatin structural changes. It is plausible to consider that a series of nuclear catalytic events need to take place both before and after the ISGF3 complex gains access to the ISG-promoter region. General models of inducible gene expression for a variety of other transcriptional regulators have shown that tightly wrapped chromatin is usually inhibitory for gene expression, likely due to physical ob-

struction of promoter-binding sites that prevent access to transcription factors, co-activators, and RNA polymerase holoenzyme subunits. In the transcriptionally inactive state, the DNA template is intimately associated with core histone subunits, which collectively make up what is called a nucleosome. To gain access to the DNA template and its promoter-enhancer elements, transcription factors may recruit one or several nuclear co-activators that regulate transcriptional activity by covalent modifications of core histones (i.e., by acetylation, phosphorylation, and methylation), or by structural changes that alter the DNA-histone association (73–75).

One class of chromatin-modifying co-activators is the histone acetyltransferases (HATs), which has been shown to potentiate the activity of several groups of transcription factors (76–81). Protein complexes with HAT activities have been tightly linked with the remodeling of chromatin by acetylation of highly conserved lysine residues on the amino-terminal tails of core histones (82, 83). The transfer of negatively-charged acetyl groups onto specific lysine residues on the positively-charged histone molecules is thought to create a repulsive force between the negative charges of the hyperacetylated histones and the chromatin template, allowing for the more relaxed chromatin-DNA conformation. The family of HAT co-activators includes the CBP/p300, GCN5, P/CAF, and p160 proteins (84). The family of histone deacetylase (HDAC) proteins catalyzes the reverse reaction, which is generally repressive for gene expression. More specific reviews of the roles of HDACs in gene transcription can be found in articles by Gray and Ekstrom (85) and by Ng and Bird (86).

Not all histone-modifying proteins are acetyltransferases. Chromatin regulation also depends on other types of histone tail modifications such as lysine and arginine methylation and tyrosine phosphorylation (87, 88). The STAT1 methyltransferase PRMT1 also modifies histones by methylation on conserved arginine residues. Another class of co-activator proteins remodel chromatin structure in adenosine triphosphate (ATP)-dependent fashion, and includes SWI/SNF and RSC proteins (75, 89, 90). These ATPases are thought to generate superhelical torsion of the DNA template, forcing the DNA apart from histones (90, 91).

Emerging evidence demonstrates that the various chromatin-modifying proteins work together in well-orchestrated fashion to activate antiviral gene expression. The systematic re-

cruitment of several HAT proteins—first GCN5, then CBP (in association with RNA polymerase II), and finally, the SWI/SNF complex—was shown to be required for the remodeling of the nucleosome structure of the IFN gene locus (92, 93).

A number of transcriptional regulators also directly or indirectly recruit co-activator proteins that primarily serve as architectural links between promoter-bound gene-specific and general transcription factors (GTFs). These proteins include integral components of the basal transcription core machinery, such as GTFs (such as TFIIA, B, D, E, F and H), the TATA-binding protein (TBP), and TBP-associated factors (TAFs). Another co-activator family is represented by the multi-protein metazoan Mediator complex. First genetically identified in yeast, the Mediator is an essential part of the RNA polymerase II holoenzyme complex that is responsible for transcription. Mediator homologues were soon found in humans and now include several multi-protein complexes, such as the thyroid hormone receptor associated protein (TRAP), vitamin D₃ receptor interacting protein(s) (DRIP), and activator-recruited cofactor (ARC) complexes (75, 94, 95). Functional interactions with the mediator complex components are mostly limited to transcriptional regulators that belong to the lipophilic nuclear receptor superfamily (such as the vitamin D and thyroid hormone receptors), and to a handful of non-nuclear receptor transcriptional regulators such as p53 and Sp-1 (75, 94–96).

Recruitment of Transcriptional Co-activators by STATs

Transcriptional regulation by STAT proteins is in large part achieved by the recruitment of general or gene-specific transcriptional co-activators by their carboxyl-terminal transcriptional activation domains (summarized in the Table). One protein that has been reported to interact with several STAT proteins at their C-terminal TADs is CBP/p300. STATs 1, 2, 5, and 6 have been shown to recruit CBP/p300 (65, 97–100). Interestingly, the STAT1 protein also interacts with CBP/p300 at its N-terminal end (97). STATs likely recruit CBP/p300 to remodel chromatin structure at target gene loci to enhance promoter accessibility for the activated STAT complexes, and possibly for additional regulators that contribute to transcriptional synergy. With respect to Types I and II IFN signaling pathways, the recruitment of the CBP/p300 protein enhances ISRE- and GAS-dependent transcriptional responses (65, 97), suggesting the importance of HAT activities in IFN responses. While the STAT2 carboxyl-terminal TAD is critically important for Type I IFN responses, it is unclear whether the STAT2-CBP/p300 interaction is the one that makes this domain indispensable. Further, while it is recognized that histones are targets for acetylation by the CBP/p300 proteins, the STATs themselves have recently been shown to be substrates as well. One recent study showed that CBP acetylation of STAT6, in addition to histones, was required for the transcription of the 15-lipoxygenase-1 (15-LOX-1) gene (101).

TABLE
Proteins That Interact with ISGF3 Components

ISGF3 Component	Interacting Partners	Comments
IRF9	STAT1, STAT2	The carboxyl-terminal STAT-binding domain (SBD, see Fig. 1) mediates contact with the coiled-coil regions of STAT1 and STAT2.
STAT1	CBP/p300, MCM5, BRCA1, Sp1, Nmi-1, TFII-I	The histone acetyltransferase CBP/p300 contacts STAT1 at both the amino-terminus and the carboxyl-terminal transcriptional activation domain (TAD). Both MCM5 and BRCA1 are recruited following serine-727 phosphorylation in the Type II IFN -dependent signaling pathway. The involvement of STAT1 co-activators in Type I IFN ISGF3-dependent signaling remains unclear.
STAT2	CBP/p300, IRF9, STAT1	CBP/p300 interacts with the carboxyl-terminal STAT2 TAD.

Thus, in addition to post-translational amino acid modifications by tyrosine/serine phosphorylation and arginine methylation, the functions of STATs are also regulated by acetylation. Also, it will be interesting to determine whether STAT1 or STAT2 is a target for acetylation, much like STAT6, under certain physiologic conditions.

For several STAT proteins bearing the PMSP/PSP phosphoserine module, phosphorylation of this highly conserved carboxyl-terminal serine is a prerequisite for the recruitment of co-activator proteins that aid STATs in enhancing gene transcription (67). For STAT1, cytokine-induced serine 727 phosphorylation leads to the direct recruitment of transcriptional co-activators. One interacting co-activator is MCM5, a member of the highly abundant minichromosome maintenance (MCM) protein family. Co-expression of STAT1 with MCM5 enhanced GAS-dependent reporter gene activity (102). STAT1-associated MCM5 in turn binds MCM3, suggesting the recruitment of a multimeric protein complex to the STAT1 TAD (103). MCM proteins, which possess DNA helicase activities thought to catalytically unwind DNA, are required for DNA replication control and are often found associated with other proteins such as the origin-replication complex at the initiation site of DNA replication. MCM proteins also interact with the RNA polymerase II holoenzyme (104), which may link helicase DNA-unwinding functions with transcriptional control in IFN-dependent gene expression. The specific nature of MCM-STAT interactions is still being investigated.

Another protein found to be recruited by the STAT1 TAD is the breast and ovarian cancer susceptibility marker BRCA1. The role of BRCA1 in DNA repair is well described, but there is emerging evidence that supports its role as a transcriptional regulator (105, 106). It has been found that BRCA1 works in conjunction with STAT1 to differentially induce an IFN- γ -responsive gene involved in growth inhibition (107). BRCA1 and STAT1 were found to synergistically induce the expression of cyclin-dependent kinase inhibitor *p21WAF1* in response to IFN stimulation. STAT1 could not activate *p21WAF1* transcription in BRCA1-deficient breast cancer cells. Importantly, the recruitment of BRCA1 by STAT1 was shown to be dependent on serine 727 phosphorylation (107). The role of BRCA1 as a co-activator for other STAT1 transcriptional activities remains unclear, but the requirement for BRCA1 in

p21WAF1 activities has important implications for tumor suppressor activities of both BRCA1 and IFN.

The list of co-activators that interact with STAT1 is certainly much larger than that of STAT2 (see Table). Thus far, CBP/p300 is the only identified STAT2 co-activator. In the Type I IFN pathway, the prevailing view is that either STAT1 or STAT2 can interact with STAT2 and IRF9 to form ISGF3, since several lines of evidence support STAT2 as the sole provider of activating signals for ISRE-dependent transcription. However, post-translational methylation of STAT1 also appears to affect Type I IFN signaling. Whether ISGF3 complexes that contain STAT1 also recruit STAT1-specific co-activators (such as MCM5 and BRCA1), and whether these complexes induce the transcription of a slightly different profile of ISGs, remain topics of current investigations.

Conclusion

A review of the STAT cell-signaling and transcription literature suggests that the mechanisms underlying the interferon responses are becoming more complex with each new finding. Critically important for Type I IFN-dependent transcriptional functions is the carboxyl-terminal transcriptional activation domain of STAT2, but the lack of characteristic regulatory features such as the PMSP phosphoserine motif means that the ISGF3 complex employs different mechanisms to activate transcription than STATs in other signaling pathways. Why are the other components of ISGF3—STAT1 and IRF9—required for ISGF3-dependent transcription? It now appears that novel post-translational modifications of STAT1, and potentially of STAT2, may also regulate Type I IFN responses. The IRF9 component may also modulate ISGF3 cell signaling through its cytoplasmic interactions with STAT2, or through STAT-independent nuclear activities. Also, still far from complete is the list of general and gene-specific co-activator proteins that interact with the ISGF3 complex to assist in antiviral gene expression. Finding additional nuclear-binding partners for ISGF3 will undoubtedly enhance our understanding of the biochemical and molecular mechanisms by which antiviral genes are switched on and off, and provide targets for pharmaceutical design.

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