

STAT2 phosphorylation and signaling

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Keywords: interferon, STAT2, phosphorylation, regulation, receptors

Abbreviations: STAT, signal transducer and activator of transcription; IFN, interferon; ISGF3, interferon-stimulated gene factor 3; CCD, coiled-coil domain; DBD, DNA-binding domain; LD, linker domain; SH2, Src homology 2; TAD, transactivation domain; IRF9, interferon regulatory factor 9; ISG, interferon stimulated gene; IFNAR, interferon alpha/beta receptor; ISRE, interferon stimulated response element

STAT2 is an essential transcription factor in type I IFN mediated anti-viral and anti-proliferative signaling. STAT2 function is regulated by tyrosine phosphorylation, which is the trigger for STAT-dimerization, subsequent nuclear translocation, and transcriptional activation of IFN stimulated genes. Evidence of additional STAT2 phosphorylation sites has emerged as well as novel roles for STAT2 separate from the classical ISGF3-signaling. This review aims to summarize knowledge of phosphorylation-mediated STAT2-regulation and future avenues of related STAT2 research.

Introduction

The signal transducer and activator of transcription (STAT) protein family was discovered in the early 1990s and quickly regarded as elegant and straightforward cytoplasmic proteins that following activation by a ligand-receptor-kinase complex translocated to the nucleus and induced gene expression.¹ STAT2 was defined as a co-factor only involved in type I IFN (IFN- α , - β , - τ , - ω) signaling, as compared with STAT1, which was found to be important in additional cytokine-induced signaling pathways, such as IFN- γ , IL-4, IL-6, and IL-27 to name a few examples.² In the past decade numerous reports have hinted at STAT2 being involved in contexts other than the classical type I IFN signaling pathway. This, together with the discovery of several novel STAT2 phosphorylation sites, prompts a summary of where we are in our current understanding of STAT2 function and regulation. This review will shed light on the mechanisms, relevance, and recent developments regarding phosphorylation-dependent STAT2 regulation.

Overview of the JAK-STAT Pathway

STAT proteins are critical for transmitting information from many different transmembrane surface receptors, such as

cytokine and hormone receptors, to the nucleus.³ The STAT family contains seven members (STAT1–6 including STAT5A and STAT5B) all of which share the same overall domain architecture: an N-terminal domain (ND), a coiled-coil domain (CCD), a DNA-binding domain (DBD), a linker domain (LD), a Src homology 2 (SH2) domain, and a transactivation domain (TAD) (Fig. 1). STATs are activated by phosphorylation of a conserved tyrosine performed by one or more receptor-associated tyrosine kinases from the Janus family of tyrosine kinases named JAK. Tyrosine phosphorylation results in reciprocal binding of one phosphorylated STAT to another, forming either a homodimer or a heterodimer. Dimer formation is the critical step for nuclear translocation since the nuclear localization signal, required for nuclear import, is formed by parts of dimerized STAT DBDs.⁴ In the nucleus, the STAT-dimer binds the promoter of genes and either induce or repress mRNA expression. In type I IFN signaling, type I IFN binds its cognate IFN receptor (IFNAR) that is pre-associated with receptor-associated kinases TYK2 and JAK1, which become activated through close proximity trans-phosphorylation (Fig. 2). This in turn causes TYK2 and JAK1 to phosphorylate the intracellular chains of the IFNARs that then serve as docking sites for STAT1 and STAT2. JAK1 and TYK2 phosphorylate STAT1 and STAT2, triggering their dimerization and association with the DNA-binding Interferon regulatory factor 9 (IRF9) resulting in the formation of the IFN stimulated gene factor 3 (ISGF3) complex. ISGF3 then binds to the promoters of IFN stimulated genes (ISGs) to activate gene expression.⁵ ISGF3 recognizes an IFN stimulated response element (ISRE) and its direct binding to DNA is mediated by STAT1 and IRF9, whereas STAT2 is responsible for recruiting transcriptional co-activators through its TAD.^{6,7} STAT1 is present as two isoforms, STAT1 α and STAT1 β .⁸ STAT1 α is the full-length form, whereas STAT1 β has a truncated and, therefore, non-functional TAD. The important TAD in ISGF3 is provided by STAT2 whereas STAT1 α and STAT1 β are interchangeable in ISGF3 signaling.^{6,9} STAT2-TAD has been shown to bind and recruit important transcriptional co-activators, such as p300/CBP, GCN5, DRIP150 and pp32.^{10–14} The biological significance of STAT2 in type I IFN signaling is apparent in STAT2 deficient mice.¹⁵ These mice are vulnerable to viral infection and host immune response is compromised. It is important to stress that mouse and human STAT2 are markedly divergent in the TAD. Mouse STAT2 contains 12 copies of a

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Submitted: 05/02/2013; Revised: 07/16/2013; Accepted: 07/17/2013
Citation: Steen HC, Gamero AM. STAT2 phosphorylation and signaling. JAK-STAT 2013; 2:e25790; <http://dx.doi.org/10.4161/jkst.25790>

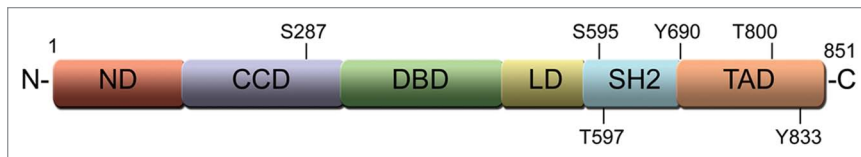


Figure 1. Schematic of the domain architecture of STAT2 and location of identified phosphorylation sites. ND, N-terminal domain; CCD, coiled-coil domain; DBD, DNA-binding domain; LD, linker domain; SH2, Src homology 2 domain; TAD, transactivation domain.

24-nucleotide mini-satellite inserted into its TAD, in addition to having flanking non-conserved sequences, and yet it is still capable of activating transcription in human cells at comparable levels to human STAT2.^{11,16} The role of STAT2 in anti-viral signaling is further supported by a recent study in which a child lacking STAT2 had a history of disseminated vaccine-strain measles.¹⁷ The deficiency in STAT2, although not lethal, was caused by a homozygous mutation in an intron donor site that prevented correct STAT2 splicing. Further analysis of common anti-viral pathways pinpointed loss of STAT2 as the most likely cause for viral sensitivity.

Activation of STAT2

In 1988, Levy et al. identified the ISRE sequence and observed a cellular factor (named ISGF3) binding to an ISRE DNA-probe in response to IFN- α treatment.¹⁸ Subsequent studies uncovered that ISGF3 consisted of four pre-existing polypeptides. Three of these peptides, with the molecular size of 84, 91, and 113 kDa, were suggested to be from the same protein family and were later named STAT1 β , STAT1 α and STAT2, respectively.^{8,19} Reich and Pfeffer observed in 1990 that the broad-spectrum kinase inhibitor staurosporine prevented IFN- α induced ISGF3 formation and ISG expression, indicating a requirement of kinase activity for IFN-signaling.²⁰ Phosphopeptide mapping confirmed that STAT2, as well as both forms of STAT1, were phosphorylated at tyrosine (Y)-690 (STAT2) and Y701 (STAT1) in response to IFN- α .²¹⁻²⁴ At that same time, a panel of IFN unresponsive mutant cell lines derived from the human fibrosarcoma cell line 2fTGH were generated and became instrumental in the characterization of the type I IFN signaling pathway.²⁵ Analysis of these non-responsive cell lines led to the identification of TYK2 and JAK1 as essential for STAT2 and STAT1 activation.^{26,27} The 2fTGH mutant cell line lacking STAT2 was named U6A and has been used in numerous studies to characterize STAT2 function. An early finding in these cells demonstrated that STAT1-activation was dependent on STAT2 in type I IFN signaling, but not in response to type II IFN (IFN- γ).^{28,29} This effect, however, is not common in all cell lines. We and others have observed that in STAT2-deficient cells other than U6A, STAT1 tyrosine phosphorylation is unaffected.^{30,31} Further studies in U6A cells have shown that the N-terminal part (1–315; includes ND and CCD) of STAT2 was critical for STAT1-activation most likely due to the pre-association of IFNAR2 with STAT2 in untreated cells.³² Together with the finding that STAT2 and STAT1 exist as dimers in resting cells, this suggested that a pre-initiation

complex of IFNAR2:STAT2:STAT1 was critical for proper type I IFN signaling.³³ Analysis of truncated and mutated versions of STAT2 in U6A cells shed several important regions and residues for proper STAT2-activation and gene induction (Table 1).⁶ Deletions from the STAT2 N-terminus disrupted tyrosine phosphorylation similarly to what was observed in the study by Li and colleagues.³² Mutation of a conserved arginine (R)-601 to lysine also abrogated STAT2 activation, thus highlighting the relevance of the SH2 domain for binding the phospho-tyrosine (pY) of the interacting partner.⁴³ Our lab has shown that mutating Y631-STAT2 to phenylalanine, which is also close to the SH2-pY interface based on the crystal structure of STAT1, prolonged STAT1-activation and ISGF3-signaling due to a decrease in STAT1 dephosphorylation.^{35,44} Y631 was not found to be phosphorylated. In contrast, mutating proline 630-STAT2 to leucine produced the opposite phenotype; it reduced ISGF3-signaling due to impaired STAT2 tyrosine phosphorylation.³⁶ The affinity of the pY for a SH2 domain is most often determined by one or more amino acids directly C-terminal of the pY.⁴⁵ Amino acid residue R694-STAT2 was found to be important since R694 substitution to asparagine caused impaired STAT2 phosphorylation.⁶ C-terminal truncations of the TAD did not affect STAT2 tyrosine phosphorylation, and yet it dramatically affected induction of ISGs. The region between amino acids 800 and 831 was critical for ISRE-mediated ISG expression, and, interestingly, also for the induction of IRF1, a STAT1-driven ISG.

Type I IFN Receptors in STAT2 Activation

The type I IFN receptor is formed by two receptor chains, IFNAR1 and IFNAR2. Both chains interact with IFN to form a ternary complex (IFN:IFNAR1:IFNAR2), with IFNAR2 binding the ligand with higher affinity compared with IFNAR1.⁴⁶ The close proximity between IFNAR1 and IFNAR2 after ligand binding facilitates reciprocal trans-phosphorylation and activation of the two receptor-associated kinases, TYK2 and JAK1. Activated TYK2 (bound to IFNAR1), and JAK1 (bound to IFNAR2) phosphorylate tyrosine residues on the receptor chains that act as docking sites for the SH2-domain of STAT2.⁴⁷⁻⁴⁹ As STAT2 binds to the receptor chain, it becomes phosphorylated on Y690 just C-terminal of the SH2-domain. The exact sites on the intracellular chains of IFNARs that STAT2 binds to are partially known. Y337 and Y512 of IFNAR2 are seemingly important because mutation of all tyrosines in IFNAR2 to phenylalanine (F) abrogated STAT2-activation.⁵⁰ Restoring F337 or F512 back to tyrosine rescued the non-functional phenotype. STAT2 was also shown to constitutively bind a stretch of acidic residues located at amino acids 435–438 of IFNAR2.^{32,51,52} This pre-association is dependent on the ND of STAT2 when examined in 2fTGH cells. The importance of this interaction is unclear because truncation of IFNAR2 or mutating amino acids 435–438, thereby disrupting the STAT2-IFNAR2 interaction, minimally affected STAT2 activation by IFN- α . The

intracellular region of IFNAR2 when overexpressed has been shown to be cleaved in a γ -secretase dependent manner in response to type I IFN, phorbol 12-myristate 13-acetate and EGF.⁵³ The proteolytically released intracellular domain (ICD) modulated the transcriptional response in several GAL4 reporter assays.^{53,54} Binding of IFNAR2-ICD to STAT2 was necessary for the transcriptional effect of the ICD, as well as the presence of an intact STAT2 TAD. Because these observations were made using exogenously expressed IFNAR2, it is unknown if endogenous IFNAR2 is cleaved during IFN signaling or to what extent IFNAR2-ICD complements type I IFN signaling.

In vitro studies have pinpointed IFNAR1 Y466 and Y481 to be phosphorylated by TYK2, and that STAT2 binds to a phosphopeptide consisting of Y466 and surrounding residues in a SH2-dependent manner.^{32,47,55} STAT2 also bound pY481, but this interaction was weaker when compared with pY466. Treatment of permeabilized HeLa cells with IFNAR1 Y466 phosphopeptide prevented IFN- α induced STAT2 and STAT1 activation. In a separate study, however, Y466F or Y481F mutations did not abolish STAT2 activation, and neither did deleting IFNAR2-404-462, but co-expressing the two altered IFNARs led to loss of STAT2 phosphorylation.⁵¹ Some studies have questioned the in vivo significance of pY466, and to a lesser extent pY481, for STAT2 activation. These two tyrosines are found in human, but not in mouse IFNAR1, and the only two conserved tyrosines, Y518 and Y529 in mouse IFNAR1 (Y527 and Y538 in human IFNAR1), were dispensable since mouse IFNAR1 truncated at amino acid 511 remained fully capable of activating STAT2.⁵⁶ The critical region in mouse IFNAR1 was confined to a section between residues 471 and 511, which is devoid of tyrosines indicating a different mechanism for STAT2-activation in mouse. This is likely given that the TAD domains of mouse and human STAT2 are 39% identical and were found to bind overlapping but also distinct proteins.¹⁶ The study of JAK1 and TYK2, and the role of each individual kinase in STAT2 tyrosine phosphorylation, has been difficult to discern due to the interdependence one kinase has on the other for activation.⁵⁷ Nonetheless, knock-down or inhibition of either JAK1 or TYK2 severely decreases tyrosine phosphorylation of STAT2.^{9,26} The final step of STAT-dimer formation is the release from the receptor-chain and binding to another STAT through SH2-pY interaction. The exact mechanism that triggers this release from the receptor chain is not known, but pY-STAT2 was reported to not associate with phosphorylated Y466 of IFNAR1, whereas unphosphorylated STAT2 did.⁴⁷ Presently, it is unknown if this is due to pY-STAT2 already binding to another STAT with higher affinity or an intrinsic change of the STAT2 SH2-domain in response to the intramolecular pY.

Deacetylation and acetylation events are important in type I IFN signaling. Early studies using the histone deacetylase inhibitor Trichostatin A demonstrated that increased global acetylation prevented proper ISGF3 function and reduced nuclear translocation of STAT2 in response to Newcastle disease virus infection.⁵⁸ In contrast, a study by Tang et al. concluded that acetylation of ISGF3 was required for proper ISGF3-dependent signaling.⁴¹ The histone acetylase CREB-binding protein was recruited to

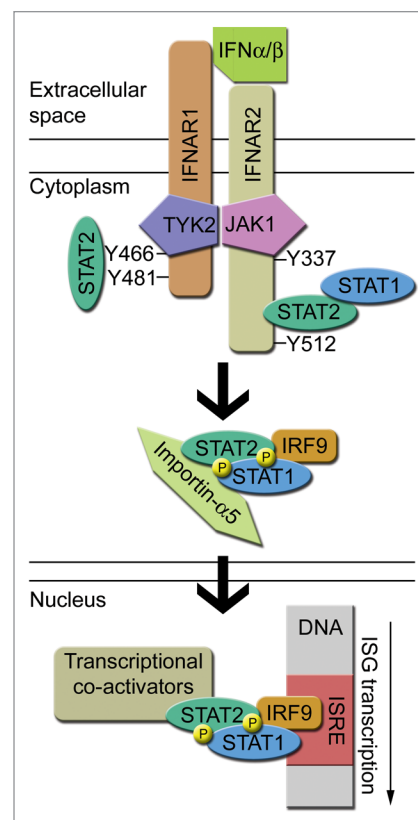


Figure 2. Illustration of type I interferon (IFN) signaling and ISGF3-mediated gene induction. Type I IFN binds IFNAR1 and IFNAR2 leading to transactivation of receptor-associated JAK kinases (JAK1 and TYK2). Several tyrosine residues on the intracellular chains of the receptor subunits are phosphorylated by JAKs, leading to recruitment and phosphorylation of STAT2 and STAT1. STAT2, STAT1, and IRF9 form the ISGF3 complex, which translocates to the nucleus by binding to importin- α 5. In the nucleus, ISGF3 binds to the interferon-stimulated response element (ISRE) of interferon-stimulated genes (ISGs) and induce gene transcription with the help of additional co-activators recruited by STAT2.

IFNAR2 in response to IFN- α resulting in the acetylation of IFNAR2, IRF9, and STAT2 on several critical lysines (K) including K399-IFNAR2, K81-IRF9, and K390-STAT2. Mutation of any of these lysines reduced ISRE reporter gene response. Acetylation of K390-STAT2 was suggested to be important for proper formation of the active STAT2:STAT1 dimer.

STAT2 Serine/Threonine Phosphorylation

Serine and threonine phosphorylations are responsible for 90% and 10% of all eukaryotic phosphorylation events; respectively, with tyrosine phosphorylation being much rarer at around 0.05%.⁵⁹ Serine phosphorylation is also involved in modulating the function of STATs. The main site for STAT serine phosphorylation, except for STAT2 and STAT6, is a conserved serine flanked by two prolines in the TAD. This site is usually situated 20–30 residues C-terminal of the pY. For STAT1, STAT3, STAT4, and STAT5B this post-translational modification (PTM) can increase the transcriptional activity of these STATs.^{60–63} In the case of STAT3, phosphorylation of Serine

Table 1. Summary of mutations that alter STAT2 signaling and function

Phenotype	STAT2 mutation	Domain	References
Prolonged Y690 phosphorylation	S287A	CCD	34
	Y631F	SH2	35
Reduced or no Y690 phosphorylation	S287D	CCD	34
	R601L	SH2	6
	P630L	SH2	36
	Y690F	C-terminus of SH2 domain	28
	R694N	C-terminus of SH2 domain	6
Reduced or no nuclear translocation	R374A + K375A	DBD	37
	R409A + K415A	DBD	37
Reduced or no nuclear export	L737A + L741A	TAD	38
	L740A + L741A	TAD	39
	L745A	TAD	39
	L751A	TAD	39
Reduced DNA-binding	V453I + V454I	DBD	40
Reduced transcriptional activity	K390R	DBD	41
Abolished STAT4 activation	Y833F	TAD	42

(S)727 can also decrease tyrosine phosphorylation.^{60,64,65} S727-STAT1 phosphorylation is necessary for STAT1 homodimers to induce a strong transcriptional response during IFN signaling. However, pS727-STAT1 is seemingly not important for ISGF3-mediated ISG induction since STAT1 β , which has a truncated TAD with a C-terminus lacking S727, is interchangeable with STAT1 α for gene expression.⁹ This raises the question of whether STAT2, as the TAD provider, is modified by additional phosphorylation events to potentiate or suppress gene expression. Several studies have hinted at or shown that STAT2 is serine or threonine phosphorylated. While mapping IFN-induced STAT2 phosphorylation, serine-phosphorylated STAT2 was detected in IFN- α stimulated cells.²² In a recent study by Ng et al. STAT2 was “phospho-shifted” as a consequence of exogenous expression of Inhibitor of nuclear factor κ -B Kinase subunit epsilon (IKK ϵ , a serine/threonine kinase), but not by catalytically inactive IKK ϵ .⁶⁶ In most recent reports, STAT2 was found phosphorylated at S595 in a phosphoproteomic screen designed to identify phosphorylation events in Jurkat cells responding to T-cell receptor activation.⁶⁷ S595, however, was not validated to be phosphorylated uniquely by T-cell receptor activation indicating the possibility that this site could be constitutively modified or phosphorylated in response to another signaling pathway. Furthermore, a phosphoproteomic analysis, as a part of the Chromosome-Centric Human Proteome Project, found threonine (T)-800 of STAT2 to be phosphorylated in human colorectal cancer tissue.⁶⁸ T597 and Y833 have also been identified as phosphorylation sites from

curated information (www.phosphosite.org).⁶⁹ In a study investigating STAT4-activation in response to type I IFN in human T-cells, the recruitment of STAT4 to the IFNAR complex was found to be STAT2-dependent.⁴² The STAT4-interacting region of STAT2 was mapped to amino acids 811–851, with Y833 being a critical residue.²⁹ The phosphorylation-status of Y833 was not assessed, but modification of Y833 may be important in STAT2-STAT4 interaction. No biological data on the importance or function of phosphorylated T597 or T800 are available at the moment.

We recently analyzed STAT2 by mass spectrometry in an attempt to find additional regulatory PTMs. Serine 287 was identified as phosphorylated in response to IFN- α treatment.³⁴ Sequence alignment revealed no conservation of S287 between human and mouse STAT2. U6A-cells reconstituted with S287A-STAT2 were found more responsive to the antiproliferative and antiviral effects of IFN- α . Biochemical analysis further revealed that Y690-phosphorylation of S287A-STAT2 was prolonged and ISG-induction was enhanced after an overnight treatment with IFN- α compared with WT-STAT2. The phosphomimetic mutant, S287D-STAT2, had the opposite effect of S287A. Expression of S287D-STAT2 reduced IFN- α -induced pY690-STAT2 and drastically decreased nuclear translocated STAT2 and consequently ISG induction. S287D-STAT2 also poorly protected cells against vesicular stomatitis virus infection. Because an antibody recognizing pS287-STAT2 is not available, the dynamics of S287 phosphorylation remain unknown at the moment. Comparison of WT-STAT2 to S287A-STAT2 and S287D-STAT2 indicates that the majority of WT-STAT2 molecules are most likely not phosphorylated on S287 since the biological and biochemical difference between WT and S287A were less than between WT and S287D. Inhibitory phosphorylation events are not unprecedented in the STAT family with the existence of such events in STAT5A, STAT5B, and STAT6.^{63,70,71} The analogous site to S287-STAT2 in STAT1 is T288. This STAT1 site is found mutated to alanine in some patients with chronic mucocutaneous candidiasis.⁷² Cells expressing this mutation had protracted responses to cytokines that depend on STAT1 for signaling. T288A-STAT1 activation was prolonged due to defects in STAT1 tyrosine dephosphorylation, and believed to be the result of the mutation restricting the STAT1-dimer to adopt a favorable conformation for dephosphorylation. The similarities between S287A-STAT2 and T288A-STAT1 suggest that phosphorylation of S287 accelerates dephosphorylation of STAT2 thus contributing to termination of JAK-STAT signaling.

Phosphorylation that negatively affects intermolecular binding between STATs is not rare. Two interesting studies from the Maniatis lab have shown that STAT1 can be phosphorylated at S708 by IKK ϵ .^{66,73} Loss of IKK ϵ reduced the expression of a subset of ISGs. The affected genes lacked a suggested STAT2-binding motif upstream of the consensus ISRE, thereby making the ISRE a lower-affinity binding site for ISGF3. Through several in vitro biochemical experiments, phosphorylation of S708-STAT1 was shown to decrease STAT1 homodimer formation due to interference of the pY701-SH2 interaction. However, formation of the STAT2:STAT1 heterodimer was not affected leading

to increased ISGF3 assembly in the presence of active IKK ϵ and higher expression of ISGs with low-affinity ISREs. There is no analogous site to S708-STAT1 in STAT2.

Unresolved Areas of STAT2 Function

The identification of several phosphorylated residues on STAT2 raises the question if the gaps in our knowledge of STAT2 could be explained by additional post-translational events. The role of STAT2 as a critical unit in ISGF3 is well established. However, multiple studies indicate a larger role for STAT2 in gene regulation beyond ISGF3. Several STAT2 complexes have been identified *in vitro*, including STAT2:STAT1 heterodimers without IRF9, STAT2 homodimers and STAT2:STAT3 heterodimers.^{7,74-76} STAT2:STAT1 and STAT2 homodimers could bind a version of gamma-activated sequence, the consensus sequence that several STAT-dimers bind to including STAT1, in gel-shift assays, but with much lower affinity than STAT1 homodimers. Careful examination of the DBD of STAT2 revealed two conserved valines (V453 and V454), previously shown to be essential in STAT5 for DNA-binding, and also found to be important in STAT2-dependent IFN- α induced growth inhibition and viral protection.⁴⁰ A STAT2:STAT1 complex, not containing IRF9 and relying on both STAT2 and STAT1 for DNA-binding, was suggested to be the complex affected by the DNA-binding deficient STAT2 mutant, but no direct evidence for this was found. The STAT2 DNA-binding deficient mutant was subsequently used for DNA microarray analysis. A small subset of ISGs (19 genes) were identified as dependent on a functional STAT2-DBD for induction in response to IFN- α .⁷⁷

STAT1-Independent STAT2 Function

The *in vivo* importance of alternative STAT2-containing dimers is not very clear, but several studies indicate that STAT2-dependent, ISGF3-independent factors are involved in type I IFN signaling. A study on STAT1-deficient mice showed that STAT2 supported the expression of ISGs.⁷⁸ In studies evaluating DNA-binding and gene activation in spleen and bone marrow-derived macrophages, STAT2 was found associated with ISG promoters (*Oas1a*, *Oas1b*, and *Irf7*) without STAT1; albeit weakly, and mRNA expression of several ISGs remained intact with no STAT1 present.⁷⁸ STAT1-independent induction of ISGs in mouse splenocytes has also been observed by Zimmerer and colleagues.⁷⁹ APOBEC3G (A3G), a cytidine deaminase important in restricting retroviral replication, was induced in response to IFN- α by STAT2 and IRF9, in the absence of STAT1.⁸⁰ Similarly to what was found with pS708-STAT1, phosphorylation (or any other PTM) could potentially direct the formation or stability of a specific STAT2-complex. STAT2 has not only been associated with the activation of IFN-target genes. Mutated p53 (p53-mut) can provide a gain-of-function phenotype in cancer cells, such as enhanced drug resistance, proliferation and cell motility. Some p53-mut phenotypes have been connected to an increase in NF- κ B2 expression.⁸¹ Although

p53-mut did not bind the NF κ B2 promoter, STAT2 and CBP were found to be enriched at the promoter in p53-mut H1299 cells compared with cells expressing WT p53. STAT2 has also been implicated in myogenic differentiation. JAK2, STAT2, and STAT3 were all important for differentiation of primary myocytes and C12C2 cells, a skeletal muscle satellite-cell-derived line, into multinucleated myotubes.⁸² The exact mechanism of action was not described, but STAT2 expression was important for reducing HGF expression and increasing IGF2 expression, two events required for myocyte differentiation.

A Role for Non-Tyrosine Phosphorylated STAT2

Several papers have uncovered novel roles for non-tyrosine-phosphorylated STATs. One of the first studies demonstrated a role for S727-STAT1, but not Y701-STAT1, in supporting the expression of caspase-1, -2, and -3.⁸³ In a more recent study, STAT3 was detected in mitochondria with a role in oxidative phosphorylation.⁸⁴ The function of STAT3 in this context was dependent on S727 instead of Y705 phosphorylation. The effect of increased levels of STAT1 protein as a result of extended IFN-treatment was studied by Cheon and Stark.⁸⁵ They concluded that non-tyrosine-phosphorylated STAT1 prolonged ISG expression of a subset of genes, primarily immune regulatory ISGs. The exact mechanism, however, remains unknown. A role for upregulated STAT2 has also been suggested in IFN- γ signaling. An ISGF3-like complex formed by non-tyrosine phosphorylated STAT2, pY701-STAT1, and IRF9 assembled after 24 h of IFN- γ treatment, and induced a subset of ISGs.⁸⁶ Furthermore, non-tyrosine-phosphorylated STAT2 was detected to be associated with a number of ISG promoters, prior to and/or after IFN- α treatment in a chromatin immunoprecipitation sequencing assay.⁸⁷ Most recently, our lab, in collaboration with the lab of Graham Foster, made the observation that NF κ B-mediated expression of pro-inflammatory cytokines required STAT2. Phosphorylation of Y690-STAT2 was dispensable because reconstitution of Y690F-STAT2 in STAT2 deficient immortalized mouse macrophages restored NF κ B transcriptional activity.⁸⁸

In conclusion, all of these observations depict a more complex picture of STAT2 function and ISG-regulation than was initially thought when STAT2 was first studied. The future of STAT2 signaling research will most likely develop alongside general transcriptional control and epigenetics research. The core of STAT2 signaling is the transcriptional regulation of genes, of which we have much more left to discover. Multimeric transcriptional complexes with interchangeable parts, repressor complexes, and cross-talk between several different promoter elements all influence and dynamically regulate gene transcription. Add to that the complex language of chromatin remodeling in the form of histone acetylation, methylation and phosphorylation, and you have the assembly of one of the most complicated and dynamic processes in nature. Therefore, identifying additional PTMs might, in the end, give us new insights into the different aspects of STAT2 function; and when deregulated, whether they are implicated in human diseases.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Acknowledgments

This work was supported by a grant from the National Cancer Institute (RO1CA140499 to AM Gamero).

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