The Role of Signal Transducer and Activator of Transcription-2 in the Interferon Response

Håkan C. Steen and Ana M. Gamero

The signal transducer and activator of transcription-2 (STAT2) was discovered as a cellular component of the DNA binding complex known as interferon (IFN) stimulated gene factor-3. Numerous studies have confirmed that STAT2 operates as a positive regulator in the transcriptional activation response elicited by IFNs. In this article, we highlight the progress made in elucidating the pivotal role of STAT2 in driving the expression of IFN-induced genes, innate antiviral immunity, apoptosis, and cancer. A better understanding of the functional role of STAT2 in the IFN response and how STAT2 is regulated will uncover new clues to its role in diseases.

Introduction

INTERFERONS (IFNs) constitute a family of multifunctional cytokines best described for their role in host defense by linking adaptive and innate immunity and exerting antiviral, antiproliferative, apoptotic, and antitumor effects. IFNs are classified into 3 types: type I (IFN- α/β), type II (IFN- γ), and type III [IFN- λ /interleukin (IL)28/IL29] (reviewed in Platanias 2005; Donnelly and Kutenko 2010). The biological responses elicited by IFNs are mediated by the transcription of multiple IFN induced genes, which is primarily dependent on the activation and nuclear translocation of signal transducer and activator of transcription (STAT) factors. IFNs activate the Janus kinase (JAK)/STAT pathway by binding to their corresponding receptor complex (IFNAR1 and IFNAR2 for type I IFNs; IFNGR1 and IFNGR2 for type II IFN; and IFNLR1 and IL10R2 for type III IFNs). STAT2 and STAT1 become activated in response to all 3 types of IFNs. JAK1 and TYK2 are preassociated with the type I and type III IFN receptor, and phosphorylate specific tyrosine residues within the receptor chains, which serve as docking sites for the recruitment of STATs (Yan and others 1996). JAKs phosphorylate a conserved tyrosine (Y) residue situated in the C-terminal region of STAT2 (Y690 in human and Y689 in mouse) and STAT1 (Y701) that, in turn, allows for STAT1 and STAT2 to interact via reciprocal SH2-phosphotyrosyl interactions (Fu 1992; Shuai and others 1993, 1994; Improtta and others 1994;). To this date, STAT2 has only been found to be activated by IFNs and urokinase (Qureshi and others 1996; Dumler and others 1999). Formation of the interferon stimulated gene factor-3 (ISGF3) complex takes place when activated STAT1:STAT2 heterodimers are released from the receptor chains to bind the DNA-binding adapter protein, IRF9. ISGF3 translocates to the nucleus and binds DNA

containing an IFN-stimulated response element (ISRE) to activate gene transcription of IFN-stimulated genes (ISGs) (Kessler and others 1990; Veals and others 1993). While STAT1 becomes serine phosphorylated in the chromatin to acquire maximal transcriptional activity, it is unknown whether serine phosphorylation also occurs in STAT2 (Wen and others 1995).

Multiple studies conducted in cell lines and in animal models demonstrate the multifaceted role of STAT2 in various biological systems. In this article, the following features of STAT2 will be covered: (i) STAT2 is vital in innate immunity; (ii) specific viruses preferentially target STAT2 to disarm the IFN antiviral response; (iii) STAT2 has dual roles as a transcriptional repressor and transcriptional activator; and (iv) STAT2 is critical in promoting IFN-induced apoptosis. Further, accumulating evidence now supports the existence of alternative STAT2 signaling pathways that are independent of STAT1.

STAT2 and Structural Domains

STAT2 was discovered as a 113 kDa protein, the largest polypeptide in the ISGF3 complex (Fu and others 1990). STAT2 is 1 of the 7 members of the STAT family as the product of the *STAT2* gene mapped to human chromosome 12 and mouse chromosome 10. While it predominantly resides in the cytoplasm, nucleocytoplasmic shuttling of STAT2 occurs in the absence of IFN stimulation (Banninger and Reich 2004). Human and murine STAT2 are highly homologous (76% identity over the first 712 amino acids), but they diverge substantially at the carboxy terminus (Park and others 1999). The transactivation domain (TAD) of mouse STAT2 is longer and contains sequences with no homology to other known proteins (Farrar and others 2000). The *STAT2* gene product

contains 6 domains: an *N*-terminal domain (NTD), a coiled-coil domain (CC), a DNA-binding domain (DBD), a linker domain (LD), a Src homology-2 domain (SH2), and a TAD. These 6 domains are conserved in all STATs.

The role of STAT2 NTD is not well defined, but various reports indicate its requirement for the tyrosine phosphorylation of STAT2 in response to type I IFNs (Qureshi and others 1996); binding of STAT2 to IFNAR2 (Li and others 1997); and cooperative binding of STAT1:STAT2 heterodimers or ISGF3 to promoters that contain tandem IFN- γ activation sequences (GAS) or ISRE elements, respectively (Li and others 1998). In addition, BRG1, a key component of the chromatin remodeling complex SWI2-SNF2, can bind to the STAT2 NTD and facilitate transcription of the ISGs IFITM1 and IFI27 (Huang and others 2002). STAT2 CC mediates protein interactions and is the domain IRF9 binds (Martinez-Moczygemba and others 1997). STAT2 DBD was described as not binding DNA as a part of ISGF3, as direct binding of ISGF3 to DNA was mediated by STAT1 and IRF9 (Bluyssen and Levy 1997). However, other studies have reported indirect evidence that STAT2 binds DNA as a part of an ISGF3-independent STAT2-containing signaling complex (Ghislain and Fish 1996; Ghislain and others 2001; Brierley and Fish 2005). Another characteristic of STAT2 DBD is that it contains a stretch of basic residues that functions as a bipartite nuclear localization signal (NLS) when paired together with the corresponding region in STAT1 after ISGF3 formation (Melen and others 2001). No function for STAT2 LD is known. STAT2 SH2 serves 2 main functions: binding to phosphorylated IFNAR1, thereby making STAT2 available for Tyk2-mediated tyrosine phosphorylation; and binding to tyrosine phosphorylated STAT1 (pSTAT1) to form an active heterodimer (Colamonici and others 1994; Gupta and others 1996; Yan and others 1996). The most C-terminal domain is the TAD, which is essential for the recruitment of transcriptional regulators. Within the STAT2 TAD lies the nuclear export signal (NES) (Banninger and Reich 2004). This leucine-rich region allows for interactions between unphosphorylated STAT2 and Crm1, which is an integral protein of the nuclear export machinery. Furthermore, nucleocytoplasmic shuttling of STAT2 is attributed to the constitutive binding of STAT2 to the NLS-containing IRF9 to transport STAT2 into the nucleus, while the STAT2 NES exports STAT2 back to the cytosol (Martinez-Moczygemba and others 1997; Lau and others 2000; Banninger and Reich 2004).

The protein structures of STAT2 and STAT2:STAT1 heterodimers remain to be solved, but based on solved STAT1 structures and biochemical data it has been demonstrated that STAT2 and STAT1 already co-exist as heterodimers in resting cells (Stancato and others 1996). The STAT dimer binds to each other in an antiparallel conformation through interactions between their NTDs and through reciprocal CC and DBD interactions (Mao and others 2005). IFN stimulation promotes a structural rearrangement in which tyrosine-phosphorylation of the 2 STATs is a critical step for the re-orientation from antiparallel to parallel conformation (Chen and others 1998).

IFN-Induced STAT2-Containing Transcriptional Complexes

In addition to ISGF3, multiple type I IFN-activated STAT2-containing signaling complexes can form. For in-

stance, the IRF9-independent STAT2:STAT1 complex was identified to bind with low affinity to a DNA-sequence similar to the GAS element (Ghislain and Fish 1996). Double mutations introduced in the STAT2 DBD (Valine 453, 454 to Isoleucine) impaired the anti-viral response of IFN- α -treated fibrosarcoma cells to encephalomyocarditis virus infection (Brierley and Fish 2005). Later studies determined that type I IFN-induced STAT2:STAT1 heterodimers stimulated the expression of a subset of IFN-sensitive genes, whose promoters contained a GAS element, which was highly dependent on STAT2 DBD (Ghislain and others 2001; Brierley and others 2006; Jia and others 2007). These findings demonstrated that STAT2 DBD is functional in IFN-induced transcriptional responses.

Other identified STAT2-containing IFN- α regulated complexes include STAT2:STAT3 heterodimers found to exist in human myeloma U266 cells (Ghislain and Fish 1996) and STAT2:STAT6 heterodimers (Gupta and others 1999), the latter being cell type specific. It is unknown whether the STAT2 containing heterodimers just described bind DNA and are transcriptionally active. However, STAT2 did inhibit IL-4 signaling through its binding to STAT6, thereby preventing STAT6 homodimer formation (Kim and Lee 2011). Interestingly, STAT2 has been shown to be constitutively associated with IRF9 independently of STAT1 (Martinez-Moczygemba and others 1997). STAT2:IRF9 heterodimers weakly bind DNA and mediate IFN-stimulated gene expression (Bluyssen and Levy 1997). They are transcriptionally functional via ISRE elements in STAT1 deficient or STAT1 knockdown cells in a cell-type specific manner (Sarkis and others 2006; Lou and others 2009).

It has been known for some time that type II IFN (IFN- γ) activates ISGF3 in murine cells with no evidence that this phenomenon occurs in human cells (Matsumoto and others 1999; Zimmermann and others 2005). A recent report, however, found for the first time that IFN- γ activates a novel ISGF3 complex termed (ISGF3^{II}) in human cells (Morrow and others 2011). What distinguishes ISGF3^{II} from the classical ISGF3 is the presence of unphosphorylated STAT2 associated with STAT1 and IRF9. Another difference is the low mRNA levels of ISGF3^{II}-dependent ISG gene expression (EIF2AK2 and IFIT3) induced by IFN- γ ; which required longer exposure to IFN- γ (at least 24 h) to be detected when compared with the levels achieved with IFN- α activated ISGF3. ISGF3^{II} is functional, as it mediated the antiviral response to IFN- γ . Figure 1 shows STAT2 containing complexes induced by each type of IFNs and the IFN responsive element they recognize.

STAT2 as a Component of the IFN-Induced Transcriptional Machinery

STAT2 assists in transcriptional induction by providing its TAD to recruit histone acetyltransferases (HATs), including GCN5 and p300/CREB binding protein (CBP) (Bhattacharya and others 1996; Paulson and others 2002). Other molecules such as the tata-box-binding protein component TAF_{II}130 and DRIP150, a subunit of the multimeric Mediator coactivator complex, also interact with STAT2 and are important in ISGF3-dependent gene transcription (Paulson and others 2002; Lau and others 2003). Furthermore, histone deacetylases (HDACs) also contribute positively to IFN-regulated gene expression (Nusinzon and Horvath 2003; Chang and others 2004). IFN- α promotes histone H4 deacetylation,

FIG. 1. Composition of multiple signal transducer and activator of transcription-2 (STAT2) containing transcriptional complexes induced by interferons (IFNs). STAT2 can associate with STAT1 and IRF9 to form interferon stimulated gene factor-3 (ISGF3) or ISGF3^{II} (in human cells). In addition, STAT2 can form heterodimers individually with either STAT1 or IRF9. Each of these complexes will bind IFN-stimulated response element (ISRE) or IFN- γ activation sequences (GAS). Not represented are STAT2:STAT3 heterodimers and STAT2:STAT6 heterodimers. Tyrosine phosphorylated STAT1 and STAT2 are marked with P.

which depends on STAT2 interacting with HDAC1 after IFN- α treatment. STAT2 binding to HDAC1 occurs in the nucleus. Most recently, pp32, a component of the inhibitor of acetyltransferase complex, was identified as another positive regulator of type I IFN induced ISG transcription (Kadota and Nagata 2011). Although pp32 was not required in the tyrosine phosphorylation or nuclear localization of STAT2 and STAT1, its association with STAT2 and STAT1 in an IFN-dependent manner was needed to enhance expression of ISGs. Knockdown of pp32, however, was not sufficient to completely suppress IFN-induced gene transcription, indicating that additional STAT2-interacting proteins can make up for the loss of pp32. Figure 2 shows proteins identified up to now to associate with STAT2 and the site of interaction if known.

As mentioned earlier, STAT2 shuttles between the cytosol and the nucleus in resting cells (Banninger and Reich 2004). The functional relevance of nuclear STAT2 in unstimulated cells remains undefined, but a recent study sheds new light on the role of unphosphorylated STAT2 in the nucleus. Testoni and others (2011b) performed chromatin immunoprecipitation (ChIP)-chip assay with anti-STAT2 antibodies on Huh7-liver cells and primary hepatocytes to

evaluate changes in STAT2-binding to chromatin in response to IFN- α . Surprisingly, several ISG promoters (70 out of 113 studied) were occupied by STAT2 before IFN- α treatment. Out of the 70 promoters that had STAT2 bound before treatment, 10 were no longer occupied with STAT2 after treatment. These 2 findings revealed the existence of a non-classical STAT2 signaling pathway. Parallel ChIP-assays conducted with anti-phosphotyrosine-STAT2 antibodies distinguished between unphosphorylated STAT2 and tyrosine-pSTAT2 bound to the ISG promoters. As predicted, the majority of promoters that gained STAT2 in response to IFN- α was positive for pSTAT2 and, therefore, functioned in accordance to the classical JAK/STAT-pathway. By integrating ChIP-chip STAT2 and pSTAT2 data, surprisingly, several ISG promoters were occupied with unphosphorylated STAT2 either before or after IFN- α treatment or both. The expression of the genes *Herc5*, *Pitx2*, and *Cav1* was affected when unphosphorylated STAT2 no longer bound to the promoter in response to IFN treatment. This finding demonstrates that nuclear unphosphorylated STAT2 can regulate positively or negatively ISG expression.

STAT2 not only promotes ISG-induction but can also suppress gene expression in response to IFNs. An example of

FIG. 2. Structural organization of STAT2 and identified interacting proteins. STAT2 contains 6 main domains: N-terminal domain (NTD); Coiled-coil domain (C-C); DNA binding domain (DBD); Linker domain (LD); Src-homology domain-2 (SH2); and Transactivation domain (TAD). Shown are key post-translational modification sites: tyrosine (Y) phosphorylation of Y690 in humans and

Y689 in mice; and acetylation of lysine (K)390 in humans. Nine proteins associate with STAT2, of which only 5 interaction sites have been mapped. Protein-protein interactions are not drawn to scale.

gene repression by STAT2 is the p53 paralog DNp73 oncogene found upregulated in multiple human cancers (Testoni and others 2011a). IFN- α treatment causes substantial chromatin remodeling of the DNp73 promoter, which contains a functional ISRE site. Recruitment of STAT2 and the polycomb group protein Ezh2 to the promoter increased histone H3K27-methylation and transcriptional repression. These recent studies clearly reveal how much there is left to decipher about the molecular mechanisms of STAT2-dependent transcriptional regulation at the chromatin level.

STAT2 in Antiviral Immunity

The generation of STAT2 knockout (KO) mice has made it possible to study in greater detail the physiological role of STAT2 (Park and others 2000). STAT2 KO mice breed and develop normally, but compared with wild-type mice, they are vulnerable to infection when challenged by various viruses, among them vesicular stomatitis virus, dengue virus (Perry and others 2011), and sindbis virus (Gil and others 2001). To evade the antiviral protective effects of IFNs, certain viruses have developed strategies to impair the IFN signaling pathway by specifically targeting STAT2. Cells expressing dengue virus replicon show loss of human STAT2 protein (Jones and others 2005). Dengue virus protein NS5 was later identified as a vital component in reducing the levels of STAT2 protein by binding STAT2 and targeting it for degradation (Ashour and others 2009). However, this viral defense mechanism is specific for human and not mouse STAT2 (Ashour and others 2010). Human STAT2/NS5 interactions have been mapped to the STAT2 CC domain. A different study, however, contradicts this finding in that NS5 does not cause STAT2 degradation but instead hinders STAT2 tyrosine phosphorylation (Mazzon and others 2009).

Members of the *Paramyxovirus* family of viruses employ a different strategy to subvert the IFN antiviral response. STAT2 associates with the V protein of hendra, measles, and nipah viruses and becomes sequestered in the cytoplasm (Rodriguez and others 2002, 2003; Ramachandran and others 2008). Measles virus (MV) and lymphocytic choriomeningitis virus (LCMV) can also suppress the antiviral effects of IFN by employing a different tactic. Rather than reducing STAT2 levels, sequestering STAT2 in the cytoplasm, or preventing its tyrosine phosphorylation, these viruses rely on IFN-activated STAT2 to suppress the immune system via inhibition of dendritic cell (DC) differentiation (Hahm and others 2005). The role of STAT2 in DC development was confirmed in STAT2 hypomorphic mutant mice, which showed suppressed antiviral responses (Chen and others 2009). In contrast, immunity against mouse cytomegalovirus (MCMV) relies on STAT2 activity independent of type I IFNs. Deletion of the antagonistic MCMV viral protein pM27 enhanced the tyrosine phosphorylation of STAT2 and antiviral responses induced by IFN- γ (Zimmermann and others 2005). Thus STAT2 remains critical in limiting viral replication and essential in innate antiviral immunity.

STAT2-Dependent STAT1-Independent Signaling Pathways

As a component of ISGF3 and other STAT2-containing signaling complexes, it is clear that STAT2 plays an essential role in the transcriptional responses to IFNs by either di-

rect or indirect association with DNA and with a strong dependence on STAT1. However, evidence continues to accumulate in that type I IFN induction of ISGs and biological outcomes can occur in a STAT2-dependent, STAT1-independent manner. In an earlier study investigating STAT1-independent IFN-signaling, IFN- α and IFN- γ were found to regulate proliferative responses differently in cells of the mononuclear phagocyte lineage. IFN- α induced an antiproliferative effect, while IFN- γ promoted proliferation on myeloid precursor cells lacking STAT1 (Gil and others 2001). Although the involvement of STAT2 in the IFN response was not part of this study, it can be postulated that STAT2 signaling complexes lacking STAT1 may have been involved in promoting the antiproliferative effects of type I IFNs. In 2005 and 2006, 2 studies were published that uncovered type I IFN-mediated STAT2-dependent signaling pathways in the absence of STAT1. In the first study, MV infection of transgenic mice expressing MV receptor human signaling lymphocytic activation molecule on DC caused immunosuppression through type I IFNs interfering with bone-marrow DC development via STAT2-dependent STAT1-independent signaling (Hahm and others 2005). MV infection of bone-marrow cells increased STAT2 protein levels as well as tyrosine phosphorylation of STAT2. Similar findings were made with LCMV. MV and LCMV induced inhibition of DC expansion, and immunosuppression was the same in wild-type and STAT1 KO mice whereas STAT2 KO mice showed normal DC development. Therefore, the proposed molecular mechanism involves type I IFN induction by MV or LCMV followed by type I IFN/STAT2 signaling. In the second study, Sarkis and others (2006) studied the transcriptional regulation of the antiviral gene APO-BEC3G (A3G; cytidine deaminase enzyme) by IFN- α in different cell types. Induction of A3G by IFN- α was detected in human liver cells and macrophages but not in the human T-cell line H9, thus indicating cell-type differences. Expression of A3G and other ISGs (PKR, ISG15, and Mx1) in Hep3B liver cells was drastically reduced when either STAT2 or IRF9 was knocked down. Surprisingly, expression of this subset of ISGs was not affected by STAT1 knockdown, revealing that IFN- α -induced STAT1 activation is not required but STAT2 activation is critical for the expression of a subset of ISGs in liver cells. In contrast, IFN- α and IFN- γ induction of ISGs was dependent on STAT1 when examined in 293T kidney cells, suggesting cell-type selectivity to the IFN transcriptional response. Recent studies continue to support the activation of IFN/STAT2 signaling pathways without dependence on STAT1. For instance, type I IFN induction of Adar1, an RNA-specific deaminase, was shown to be upregulated via STAT2 activation in STAT1 KO mouse embryonic fibroblasts (George and others 2008). In a different report, Perry and others (2011) expanded on the earlier observations made by Gil and others (2001) in which mice lacking STAT1 were more resistant to sindbis virus and murine cytomegalovirus than IFNAR1- and IFNGR1-deficient mice. In this study, resistance to dengue virus (serotype 2 strain S221) infection and mortality among WT, STAT1KO, STAT2KO, and STAT1/STAT2 double KO mice were compared. WT and single KO mice remained resistant to virus infection. In contrast, the antiviral effect of IFN was abolished in STAT1/IFNAR1 double KO mice leaving STAT2 as the presumed signaling factor involved in the antiviral response observed in STAT1 KO mice. Gene

expression analysis of 84 known ISGs showed a group of genes that were induced in the spleens isolated from STAT1 KO mice after dengue infection, of which a subset of them was not expressed in the spleens of STAT1/STAT2 double KO mice, indicating a requirement of STAT2, but not STAT1, in mediating induction of those genes. A similar pattern was reproduced in bone-marrow-derived macrophages (BMM) established from these animals and infected with dengue virus, which employs cells of monocyte/macrophage lineage for its replication. Some of the IFN-induced antiviral genes not expressed in STAT1/STAT2 double KO mice were the same genes mentioned earlier to be induced without dependence on STAT1 (ie, *OAS1* and *MX1*) (Hahm and others 2005; Sarkis and others 2006). Further studies demonstrated that in STAT1 KO BMM, dengue infection performed in vitro did not interfere with the tyrosine phosphorylation and nuclear translocation of STAT2. ChIP assays confirmed that after dengue infection, STAT2 associated with the promoters of the *OAS1* and *IRF1* genes. What remains undetermined is whether IRF9 participates in the activation of STAT1-independent genes as a part of the STAT2:IRF9 complex or whether STAT2 binds DNA directly with additional unidentified proteins. Thus far, these data suggest that STAT1 and STAT2 can function independently in the regulation of a subset of ISGs.

STAT2 Regulation

STAT2 is ubiquitously expressed in most cell types, which most likely is an indicator of the importance of IFN signaling in the innate response against viral infections. STAT2 contains a weak ISRE in its promoter region that triggers up-regulation of STAT2 during type I IFN signaling (Yan and others 1995). The importance of an increase in STAT2 expression during type I IFN signaling has not been determined. However, STAT1 up-regulation during IFN signaling has proved to be important for prolonging the induction of ISGs, and a similar role for STAT2 up-regulation could be feasible (Cheon and Stark 2009).

STAT2 can be negatively regulated by the small G-protein Ras. Constitutively activated Ras, due to mutations in its N-terminal region, is often found in tumor cells and has in several studies been shown to inhibit the cellular response to type I IFN (Klampfer and others 2003; Battcock and others 2006; Noser and others 2007; Christian and others 2009). In the human colon carcinoma cell line HCT116, activated Ras suppressed STAT2 mRNA and protein expression, as well as STAT2-inducibility in response to IFN- γ (Klampfer and others 2003). Mouse NIH3T3 fibroblasts expressing a constitutively active Ras had compromised type I IFN signaling and anti-viral protection due to a decrease in STAT2 mRNA and protein expression (Battcock and others 2006; Christian and others 2009). Inhibition of MEK2, a mitogen-activated protein kinase functioning downstream of Ras, restored STAT2 protein expression and anti-viral efficiency of type I IFN (Christian and others 2009). The events downstream of MEK2 leading to suppression of STAT2 were not investigated and remains unknown.

Acetylation is a post-translational modification that affects chromatin remodeling and, consequently, gene expression. The consensus view on acetylation in IFN- α signaling has been that a general decrease in acetylation is beneficial for ISG induction (Nusinzon and Horvath 2003; Chang and others 2004). Tang and others (2007) presented evidence

that, in response to IFN- α , the histone acetylase CBP is recruited to IFNAR2 leading to subsequent acetylation of IFNAR2, IRF9, and STAT2 on multiple lysine (K) amino residues. Acetylation of K390 located in the DBD of STAT2 was suggested to destabilize the anti-parallel STAT2:STAT1 heterodimer that exists in untreated cells to facilitate the forming of the parallel STAT2:STAT1 heterodimer after tyrosine phosphorylation. Mutation of STAT2-K390 to arginine increased binding of STAT2 to STAT1 in untreated cells, and also disrupted induction of ISGs in response to IFN- α . So far, this is the only study that has reported on the importance of CBP-mediated STAT2 acetylation during ISGF3-induction.

STAT2 in the Antigrowth and Apoptotic Effects of IFNs

In addition to their antiviral effects, IFNs can also inhibit cell growth and even trigger an apoptotic response. STAT2, as a part of ISGF3, has been shown to play a critical role in these processes. STAT2 is important for the induction of apoptosis in human H123-cells, a Jurkat T-cell subline, as loss of STAT2 protected these cells from IFN- α -induced apoptosis (Romero-Weaver and others 2010). The exact mechanism of IFN- α -induced apoptosis is not fully clear, but in the case of H123-cells, apoptosis was promoted by a loss of mitochondrial membrane potential and caspase-3 activation. A possible involvement of STAT2 in the induction of apoptosis by other agents has not been addressed directly, but as observed by Du and others (2009), a Daudi subline lacking STAT2 was resistant not only to the apoptotic-inducing effects of type I IFNs but also to camptothecin, staurosporine, and doxorubicin, indicating that STAT2 is involved in apoptosis signaling outside its role in the JAK/STAT-pathway.

Although an apoptotic phenotype is sometimes observed in cells treated with type I IFNs, the majority of cells exposed to type I IFN respond with a decreased proliferation rate. The molecular mechanisms that determine how a cell chooses its fate have not been identified. One hypothesis is that the strength and duration of the signal triggered by IFN, in the form of ISGF3-mediated ISG-induction, may determine induction of apoptosis. This is supported by Scarzello and others (2007), where a STAT2-Y631F mutant showed prolonged STAT2 activation together with STAT1 in response to type I IFN, which coincided with increased cell death compared with cells expressing only wild type STAT2.

A link between STAT2 and tumorigenesis was observed in transgenic mice expressing constitutively IFN- α in the brain (Wang and others 2003). Under these conditions, STAT2 KO mice developed tumors in the brain. The production of IFN- γ and STAT1-dependent expression of the sonic hedgehog gene, implicated in the genesis of medulloblastoma (that was not detected in STAT1KO), was presumed to have led to the formation of tumors. Therefore, a loss of STAT2 in the continuous presence of type I IFN has adverse effects. Interestingly, mouse cells devoid of STAT2 have been shown to respond to IFN- α in an IFN- γ like manner by inducing the expression of an IFN- γ regulated gene that is not induced by IFN- α in wild-type cells (Zhao and others 2007). In a different study, STAT2KO mice were subjected to chemical-induced carcinogenesis and found to develop fewer tumors when compared with wild-type mice (Romero-Weaver and others 2010). Whether the protective effect of STAT2 deficiency is dependent on IFN signaling has yet to be determined.

Concluding Remarks

It is well recognized that STAT2 is an essential component in the transcriptional responses to IFNs. Several studies confirm that a deficiency in STAT2 has detrimental effects to the host, as this affects the biological outcomes of IFNs. As reviewed here, our initial view of how STAT2 regulates IFN induced gene transcription is changing. Not all the effects of STAT2 are dependent on STAT1. STAT2 can form heterodimers with other STATs and associate with additional transcription factors to promote ISG transcription. A most surprising discovery is that unphosphorylated STAT2 binds a subset of ISG promoters. Release of unphosphorylated STAT2 from the chromatin in response to IFN stimulation can either suppress or induce gene transcription. The mechanism of how unphosphorylated STAT2 regulates gene transcription remains to be defined. While significant progress has been made in understanding the biological role of STAT2, many aspects of STAT2 remain to be elucidated. For instance, it is unclear how STAT2 transcriptional activity is switched off. The identity of the protein tyrosine phosphatase that selectively dephosphorylates STAT2 remains unknown. Are there additional post-translational modifications occurring in STAT2 in response to IFN treatment, such as serine phosphorylation and lysine acetylation, that selectively modulate transcriptional responses to IFNs? Moreover, it has yet to be determined whether single-nucleotide polymorphisms (SNPs) in STAT2 influence IFN signaling. This information is critical, because STAT2 SNPs may influence the therapeutic effects IFNs, which are currently used in the treatment of a variety of diseases. Future studies are also expected to elucidate the precise role of STAT2 in tumorigenesis and whether the presence or absence of STAT2 may be implicated in tumor development.

Acknowledgments

The authors thank Chanyu Yue and Kevin Kotredes for their critical reading of this article.

Author Disclosure Statement

There are no competing financial interests in connection with this article to be reported.

References

- Ashour J, Laurent-Rolle M, Shi PY, Garcia-Sastre A. 2009. NS5 of dengue virus mediates STAT2 binding and degradation. *J Virol* 83(11):5408–5418.
- Ashour J, Morrison J, Laurent-Rolle M, Belicha-Villanueva A, Plumlee CR, Bernal-Rubio D, Williams KL, Harris E, Fernandez-Sesma A, Schindler C, Garcia-Sastre A. 2010. Mouse STAT2 restricts early dengue virus replication. *Cell Host Microbe* 8(5):410–421.
- Banninger G, Reich NC. 2004. STAT2 nuclear trafficking. *J Biol Chem* 279(38):39199–39206.
- Battcock SM, Collier TW, Zu D, Hirasawa K. 2006. Negative regulation of the alpha interferon-induced antiviral response by the Ras/Raf/MEK pathway. *J Virol* 80(9):4422–4430.
- Bhattacharya S, Eckner R, Grossman S, Oldread E, Arany Z, D'Andrea A, Livingston DM. 1996. Cooperation of Stat2 and p300/CBP in signalling induced by interferon-alpha. *Nature* 383(6598):344–347.
- Bluyssen HA, Levy DE. 1997. Stat2 is a transcriptional activator that requires sequence-specific contacts provided by stat1 and p48 for stable interaction with DNA. *J Biol Chem* 272(7):4600–4605.
- Brierley MM, Fish EN. 2005. Functional relevance of the conserved DNA-binding domain of STAT2. *J Biol Chem* 280(13):13029–13036.
- Brierley MM, Marchington KL, Jurisica I, Fish EN. 2006. Identification of GAS-dependent interferon-sensitive target genes whose transcription is STAT2-dependent but ISGF3-independent. *Febs J* 273(7):1569–1581.
- Chang HM, Paulson M, Holko M, Rice CM, Williams BR, Marie I, Levy DE. 2004. Induction of interferon-stimulated gene expression and antiviral responses require protein deacetylase activity. *Proc Natl Acad Sci U S A* 101(26):9578–9583.
- Chen L-S, Wei P-C, Liu T, Kao C-H, Pai L-M, Lee C-K. 2009. STAT2 hypomorphic mutant mice display impaired dendritic cell development and antiviral response. *J Biomed Sci* 16(1):22.
- Chen X, Vinkemeier U, Zhao Y, Jeruzalmi D, Darnell JE Jr., Kuriyan J. 1998. Crystal structure of a tyrosine phosphorylated STAT-1 dimer bound to DNA. *Cell* 93(5):827–839.
- Cheon H, Stark GR. 2009. Unphosphorylated STAT1 prolongs the expression of interferon-induced immune regulatory genes. *Proc Natl Acad Sci U S A* 106(23):9373–9378.
- Christian SL, Collier TW, Zu D, Licursi M, Hough CM, Hirasawa K. 2009. Activated Ras/MEK inhibits the antiviral response of alpha interferon by reducing STAT2 levels. *J Virol* 83(13):6717–6726.
- Colamonici O, Yan H, Domanski P, Handa R, Smalley D, Mulersman J, Witte M, Krishnan K, Krolewski J. 1994. Direct binding to and tyrosine phosphorylation of the alpha subunit of the type I interferon receptor by p135tyk2 tyrosine kinase. *Mol Cell Biol* 14(12):8133–8142.
- Donnelly RP, Kotenko SV. 2010. Interferon-lambda: a new addition to an old family. *J Interferon Cytokine Res* 30(8):555–564.
- Du Z, Fan M, Kim JG, Eckerle D, Lothstein L, Wei L, Pfeffer LM. 2009. Interferon-resistant Daudi cell line with a Stat2 defect is resistant to apoptosis induced by chemotherapeutic agents. *J Biol Chem* 284(41):27808–27815.
- Dumler I, Kopmann A, Wagner K, Mayboroda OA, Jerke U, Dietz R, Haller H, Gulba DC. 1999. Urokinase induces activation and formation of Stat4 and Stat1-Stat2 complexes in human vascular smooth muscle cells. *J Biol Chem* 274(34):24059–24065.
- Farrar JD, Smith JD, Murphy TL, Leung S, Stark GR, Murphy KM. 2000. Selective loss of type I interferon-induced STAT4 activation caused by a minisatellite insertion in mouse Stat2. *Nat Immunol* 1(1):65–69.
- Fu XY. 1992. A transcription factor with SH2 and SH3 domains is directly activated by an interferon alpha-induced cytoplasmic protein tyrosine kinase(s). *Cell* 70(2):323–335.
- Fu XY, Kessler DS, Veals SA, Levy DE, Darnell JE Jr. 1990. ISGF3, the transcriptional activator induced by interferon alpha, consists of multiple interacting polypeptide chains. *Proc Natl Acad Sci U S A* 87(21):8555–8559.
- George CX, Das S, Samuel CE. 2008. Organization of the mouse RNA-specific adenosine deaminase Adar1 gene 5'-region and demonstration of STAT1-independent, STAT2-dependent transcriptional activation by interferon. *Virology* 380(2):338–343.
- Ghislain JJ, Fish EN. 1996. Application of genomic DNA affinity chromatography identifies multiple interferon- α -regulated Stat2 complexes. *J Biol Chem* 271(21):12408–12413.
- Ghislain JJ, Wong T, Nguyen M, Fish EN. 2001. The interferon-inducible Stat2:Stat1 heterodimer preferentially binds *in vitro*

- to a consensus element found in the promoters of a subset of interferon-stimulated genes. *J Interferon Cytokine Res* 21(6): 379–388.
- Gil MP, Bohn E, O'Guin AK, Ramana CV, Levine B, Stark GR, Virgin HW, Schreiber RD. 2001. Biologic consequences of Stat1-independent IFN signaling. *Proc Natl Acad Sci* 98(12): 6680–6685.
- Gupta S, Jiang M, Pernis AB. 1999. IFN- α Activates Stat6 and leads to the formation of Stat2:Stat6 complexes in B cells. *J Immunol* 163(7):3834–3841.
- Gupta S, Yan H, Wong LH, Ralph S, Krolewski J, Schindler C. 1996. The SH2 domains of Stat1 and Stat2 mediate multiple interactions in the transduction of IFN- α signals. *Embo J* 15(5):1075–1084.
- Hahn B, Trifilo MJ, Zuniga EI, Oldstone MB. 2005. Viruses evade the immune system through type I interferon-mediated STAT2-dependent, but STAT1-independent, signaling. *Immunity* 22(2):247–257.
- Huang M, Qian F, Hu Y, Ang C, Li Z, Wen Z. 2002. Chromatin-remodelling factor BRG1 selectively activates a subset of interferon- α -inducible genes. *Nat Cell Biol* 4(10):774–781.
- Improta T, Schindler C, Horvath CM, Kerr IM, Stark GR, Darnell JE Jr. 1994. Transcription factor ISGF-3 formation requires phosphorylated Stat91 protein, but Stat113 protein is phosphorylated independently of Stat91 protein. *Proc Natl Acad Sci U S A* 91(11):4776–4780.
- Jia D, Rahbar R, Fish EN. 2007. Interferon-inducible Stat2 activation of JUND and CLDN4: mediators of IFN responses. *J Interferon Cytokine Res* 27(7):559–566.
- Jones M, Davidson A, Hibbert L, Gruenwald P, Schlaak J, Ball S, Foster GR, Jacobs M. 2005. Dengue virus inhibits alpha interferon signaling by reducing STAT2 expression. *J Virol* 79(9):5414–5420.
- Kadota S, Nagata K. 2011. pp32, an INHAT component, is a transcription machinery recruiter for maximal induction of IFN-stimulated genes. *J Cell Sci* 124(Pt 6):892–899.
- Kessler DS, Veals SA, Fu X-Y, Levy DS. 1990. Interferon- α regulates nuclear translocation and DNA-binding affinity of ISGF3, a multimeric transcriptional activator. *Genes Dev* 4: 1753–1765.
- Kim SH, Lee CE. 2011. Counter-regulation mechanism of IL-4 and IFN- α signal transduction through cytosolic retention of the pY-STAT6:pY-STAT2:p48 complex. *Eur J Immunol* 41(2):461–472.
- Klampfer L, Huang J, Corner G, Mariadason J, Arango D, Sasazuki T, Shirasawa S, Augenlicht L. 2003. Oncogenic Ki-ras inhibits the expression of interferon-responsive genes through inhibition of STAT1 and STAT2 expression. *J Biol Chem* 278(47):46278–46287.
- Lau JF, Nuszon I, Burakov D, Freedman LP, Horvath CM. 2003. Role of metazoan mediator proteins in interferon-responsive transcription. *Mol Cell Biol* 23(2):620–628.
- Lau JF, Parisien JP, Horvath CM. 2000. Interferon regulatory factor subcellular localization is determined by a bipartite nuclear localization signal in the DNA-binding domain and interaction with cytoplasmic retention factors. *Proc Natl Acad Sci U S A* 97(13):7278–7283.
- Li X, Leung S, Burns C, Stark GR. 1998. Cooperative binding of Stat1-2 heterodimers and ISGF3 to tandem DNA elements. *Biochimie* 80(8–9):703–710.
- Li X, Leung S, Kerr IM, Stark GR. 1997. Functional subdomains of STAT2 required for preassociation with the alpha interferon receptor and for signaling. *Mol Cell Biol* 17(4):2048–2056.
- Lou YJ, Pan XR, Jia PM, Li D, Xiao S, Zhang ZL, Chen SJ, Chen Z, Tong JH. 2009. IFR-9/STAT2 functional interaction drives retinoic acid-induced gene G expression independently of STAT1. *Cancer Res* 69(8):3673–3680.
- Mao X, Ren Z, Parker GN, Sondermann H, Pastorello MA, Wang W, McMurray JS, Demeler B, Darnell JE Jr., Chen X. 2005. Structural bases of unphosphorylated STAT1 association and receptor binding. *Mol Cell* 17(6):761–771.
- Martinez-Moczygemba M, Gutch MJ, French DL, Reich NC. 1997. Distinct STAT structure promotes interaction of STAT2 with the p48 subunit of the interferon- α -stimulated transcription factor ISGF3. *J Biol Chem* 272(32): 20070–20076.
- Matsumoto M, Tanaka N, Harada H, Kimura T, Yokochi T, Kitagawa M, Schindler C, Taniguchi T. 1999. Activation of the transcription factor ISGF3 by interferon- γ . *Biol Chem* 380(6):699–703.
- Mazzon M, Jones M, Davidson A, Chain B, Jacobs M. 2009. Dengue virus NS5 inhibits interferon- α signaling by blocking signal transducer and activator of transcription 2 phosphorylation. *J Infect Dis* 200(8):1261–1270.
- Melen K, Kinnunen L, Julkunen I. 2001. Arginine/lysine-rich structural element is involved in interferon-induced nuclear import of STATs. *J Biol Chem* 276(19):16447–16455.
- Morrow AN, Schmeisser H, Tsuno T, Zoon KC. 2011. A novel role for IFN-stimulated gene factor 3II in IFN- γ signaling and induction of antiviral activity in human cells. *J Immunol* 186(3):1685–1693.
- Noser JA, Mael AA, Sakuma R, Ohmine S, Marcato P, Lee PW, Ikeda Y. 2007. The RAS/Raf1/MEK/ERK signaling pathway facilitates VSV-mediated oncolysis: implication for the defective interferon response in cancer cells. *Mol Ther* 15(8):1531–1536.
- Nuszon I, Horvath CM. 2003. Interferon-stimulated transcription and innate antiviral immunity require deacetylase activity and histone deacetylase 1. *Proc Natl Acad Sci U S A* 100(25):14742–14747.
- Park K, Lecomte MJ, Schindler C. 1999. Murine Stat2 is uncharacteristically divergent. *Nucleic Acids Res* 27:4191–4199.
- Park C, Li S, Cha E, Schindler C. 2000. Immune response in Stat2 knockout mice. *Immunity* 13(6):795–804.
- Paulson M, Press C, Smith E, Tanese N, Levy DE. 2002. IFN-Stimulated transcription through a TBP-free acetyltransferase complex escapes viral shutoff. *Nat Cell Biol* 4(2):140–147.
- Perry ST, Buck MD, Lada SM, Schindler C, Shresta S. 2011. STAT2 Mediates Innate Immunity to Dengue Virus in the Absence of STAT1 via the Type I Interferon Receptor. *PLoS Pathog* 7(2):e1001297.
- Platanias LC. 2005. Mechanisms of type-I- and type-II-interferon-mediated signalling. *Nat Rev Immunol* 5(5):375–386.
- Qureshi SA, Leung S, Kerr IM, Stark GR, Darnell JE Jr. 1996. Function of Stat2 protein in transcriptional activation by alpha interferon. *Mol Cell Biol* 16(1):288–293.
- Ramachandran A, Parisien JP, Horvath CM. 2008. STAT2 is a primary target for measles virus V protein-mediated alpha/beta interferon signaling inhibition. *J Virol* 82(17):8330–8338.
- Rodriguez JJ, Parisien JP, Horvath CM. 2002. Nipah virus V protein evades alpha and gamma interferons by preventing STAT1 and STAT2 activation and nuclear accumulation. *J Virol* 76(22):11476–11483.
- Rodriguez JJ, Wang L-F, Horvath CM. 2003. Hendra virus V protein inhibits interferon signaling by preventing STAT1 and STAT2 nuclear accumulation. *J Virol* 77(21):11842–11845.
- Romero-Weaver AL, Wang HW, Steen HC, Scarzello AJ, Hall VL, Sheikh F, Donnelly RP, Gamero AM. 2010. Resistance to IFN- α -induced apoptosis is linked to a loss of STAT2. *Mol Cancer Res* 8(1):80–92.

- Sarkis PT, Ying S, Xu R, Yu XF. 2006. STAT1-independent cell type-specific regulation of antiviral APOBEC3G by IFN- α . *J Immunol* 177(7):4530–4540.
- Scarzello AJ, Romero-Weaver AL, Maher SG, Veenstra TD, Zhou M, Qin A, Donnelly RP, Sheikh F, Gamero AM. 2007. A Mutation in the SH2 domain of STAT2 prolongs tyrosine phosphorylation of STAT1 and promotes type I IFN-induced apoptosis. *Mol Biol Cell* 18(7):2455–2462.
- Shuai K, Horvath CM, Huang LH, Qureshi SA, Cowburn D, Darnell JE Jr. 1994. Interferon activation of the transcription factor Stat91 involves dimerization through SH2-phosphotyrosyl peptide interactions. *Cell* 76(5):821–828.
- Shuai K, Stark GR, Kerr IM, Darnell JE Jr. 1993. A single phosphotyrosine residue of Stat91 required for gene activation by interferon- γ . *Science* 261(5129):1744–1746.
- Stancato LF, David M, Carter-Su C, Lerner AC, Pratt WB. 1996. Preassociation of STAT1 with STAT2 and STAT3 in separate signalling complexes prior to cytokine stimulation. *J Biol Chem* 271(8):4134–4137.
- Tang X, Gao J-S, Guan Y-j, McLane KE, Yuan Z-L, Ramratnam B, Chin YE. 2007. Acetylation-dependent signal transduction for type I interferon receptor. *Cell* 131(1):93–105.
- Testoni B, Schinzari V, Guerrieri F, Gerbal-Chaloin S, Blandino G, Levvero M. 2011a. p53-paralog DNp73 oncogene is repressed by IFN[α]/STAT2 through the recruitment of the Ezh2 polycomb group transcriptional repressor. *Oncogene* 30(23):2670–2678.
- Testoni B, Vollenkle C, Guerrieri F, Gerbal-Chaloin S, Blandino G, Levvero M. 2011b. Chromatin dynamics of gene activation and repression in response to interferon alpha (IFN(α)) reveal new roles for phosphorylated and unphosphorylated forms of the transcription factor STAT2. *J Biol Chem* 286(23):20217–20227.
- Veals SA, Santa Maria T, Levy DE. 1993. Two domains of ISGF3 γ that mediate protein-DNA and protein-protein interactions during transcription factor assembly contribute to DNA-binding specificity. *Mol Cell Biol* 13(1):196–206.
- Wang J, Pham-Mitchell N, Schindler C, Campbell IL. 2003. Dysregulated Sonic hedgehog signaling and medulloblastoma consequent to IFN- α -stimulated STAT2-independent production of IFN- γ in the brain. *J Clin Invest* 112(4):535–543.
- Wen Z, Zhong Z, Darnell JE Jr. 1995. Maximal activation of transcription by Stat1 and Stat3 requires both tyrosine and serine phosphorylation. *Cell* 82(2):241–250.
- Yan H, Krishnan K, Greenlund AC, Gupta S, Lim JT, Schreiber RD, Schindler CW, Krolewski JJ. 1996. Phosphorylated interferon- α receptor 1 subunit (IFN α R1) acts as a docking site for the latent form of the 113 kDa STAT2 protein. *EMBO J* 15(5):1064–1074.
- Yan R, Qureshi S, Zhong Z, Wen Z, Darnell JE Jr. 1995. The genomic structure of the STAT genes: multiple exons in co-incident sites in Stat1 and Stat2. *Nucleic Acids Res* 23(3):459–463.
- Zhao W, Cha EN, Lee C, Park CY, Schindler C. 2007. Stat2-dependent regulation of MHC class II expression. *J Immunol* 179(1):463–471.
- Zimmermann A, Trilling M, Wagner M, Wilborn M, Bubic I, Jonjic S, Koszinowski U, Hengel H. 2005. A cytomegaloviral protein reveals a dual role for STAT2 in IFN- γ signaling and antiviral responses. *J Exp Med* 201(10):1543–1553.

Address correspondence to:

Dr. Ana M. Gamero

Department of Biochemistry

Temple University School of Medicine

3440 N. Broad St.

Philadelphia, PA 19140

E-mail: gameroa@temple.edu

Received 22 September 2011 / Accepted 3 October 2011