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Distinct Phosphorylation of STAT1 Confers Distinct DNA Binding and Gene-regulatory Properties

Hozaifa Metwally, Tadamitsu Kishimoto*

Laboratory of Immune Regulation, Immunology Frontier Research Center, Osaka University, 565-0871 Osaka, Japan

*Correspondence should be addressed to Tadamitsu Kishimoto, kishimoto@ifrec.osaka-u.ac.jp

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Abstract

Signal transducer and activator of transcription 1 (STAT1) protein plays a pivotal role in various biological processes especially the regulation of innate and adaptive immune responses. Phosphorylation represents a key step in the activation of STAT1 and its transcriptional outcome. Binding of various extracellular ligands to their specific cell-surface receptors activates different phosphorylation of STAT1 followed by a distinct change of gene expression patterns. STAT1 is well-studied for its role in conducting the transcriptional response to interferons (IFNs), where it is activated by Janus kinase (JAK)–mediated phosphorylation of its Tyr^{701} residue. However, the STAT1 function expands beyond its Tyr^{701} phosphorylation. In this regard, we demonstrated that STAT1 serves as a proinflammatory effector downstream of toll-like receptor 4 (TLR4) endocytosis independently of IFN-β signaling. In human macrophages, lipopolysaccharide (LPS)–bound TLR4 endocytosis activated noncanonical phosphorylation of STAT1 at Thr^{749}, which altered its DNA target motif. Thr^{749}–phosphorylated STAT1 promoted the expression of the gene encoding AT-rich interactive domain-containing protein 5A (ARID5A), which stabilizes interleukin 6 (*IL6)* mRNA. Moreover, Thr^{749}–phosphorylated STAT1 directly enhanced the transcription of the gene encoding *IL12B*. By altering its DNA binding specificity, Thr^{749} phosphorylation confers STAT1 with proinflammatory properties in LPS-stimulated macrophages independent of its Tyr^{701} phosphorylation. Thus, our study highlights the importance of understanding STAT1 phosphorylation status on its DNA binding specificity and transcriptional outcome, which may help in developing better therapeutic or diagnostic strategies targeting STAT1.

Keywords: STAT1; Phosphorylation; Thr749, Tyr701

Commentary

Signal transducer and activator of transcription (STAT) proteins have been recognized about three decades ago for their dual function as signal transducers and transcriptional activators [1-3]. In mammals, there are seven STAT proteins; STAT1, STAT2, STAT3, STAT4, STAT5a, STAT5b, and STAT6 that are involved in multiple signaling pathways regulating a wide-range of biological consequences such as host defense, development, cell growth and homeostasis [4]. For example, our group has identified STAT3 as the primary signaling transducer of interleukin 6 (IL-6) [5], a pleotropic cytokine regulating a wide range of biological processes such as hematopoiesis, apoptosis, inflammatory responses and metabolism [6,7]. Each of the STAT proteins remains latent in the cytoplasm until activated by specific extracellular proteins (primarily cytokines and growth factors) bound to their specific cell-surface receptors, culminating in differential signaling processing and transcriptional outcome [8]. All STAT proteins share conserved functional domains: N-terminal, DNA-binding, linker, Src-homology 2 (SH2), and C-terminal transactivation domain (TAD) [9,10]. The basic model for STAT proteins activation depends on binding of extracellular ligand resulting in active conformational change of the cytoplasmic domain of their specific receptors leading to the activation of various tyrosine kinases, which in turn phosphorylate a key tyrosine residue located at the region between the SH2 and the TAD (around residue 700) of STAT proteins resulting in STAT-STAT dimerization [10]. Among the well-studied STAT tyrosine kinases are the Janus kinases (JAKs), which comprise four members (JAK1, JAK2, JAK3 and TYK2) that are ubiquitously expressed and function in interferons (IFNs) and various cytokines signaling [11-15]. Given the large number of extracellular ligands compared to the small number of STAT proteins, it is evident that differential activation of multiple STATs by a single ligand or differential activation of a single STAT protein by two different ligands occur.

This paradigm is clear in case of STAT1, which is involved in conducting the signaling of IFNs culminating in the transcription of interferon stimulated genes (ISGs) and shaping an antiviral response. There are three different classes of IFN and they signal through distinct cell-surface receptor complexes: type I IFN binds to IFN-α receptor 1 (IFNAR1) and IFNAR2 heterodimer; type III IFN binds to IL-10 receptor 2 (IL-10R2) and IFN-λ receptor 1 (IFNLR1) heterodimers; and type II IFN binds to IFN-γ receptor 1 (IFNGR1) and IFNGR2 heterodimer [16]. Type II IFN activates JAK1/JAK2 kinase complex, which in turn promotes tyrosine phosphorylation at residue 701 (Tyr^{701}) of STAT1, followed by its homodimerization, nuclear translocation and transcriptional activation of IFN-γ–induced genes through binding at gamma-activated sequence (GAS) of their DNA promoter regions [17-20]. On the other hand, type I and III IFNs activates JAK1/TYK2 kinase complex, which promotes tyrosine phosphorylation of both STAT1 (Tyr^{701}) and STAT2 (Tyr^{690}) leading to their heterodimerization and association with IFN regulatory factor 9 (IRF9) forming the ISG factor 3 (ISGF3) complex that activates the transcription of multiple ISGs through binding to IFN-stimulated regulatory elements (ISREs) in their DNA promoter regions [21-26]. Intriguingly, the GAS sequence is not only activated by STAT1, but it is also shared among all other STATs except for STAT2 with variable gene expression pattern mediated by each STAT protein [27], denoting that the relative abundance STAT proteins and their DNA binding partners play important role in shaping their transcriptional outcome. For example, IL-21 activates opposing functions of STAT1 and STAT3 in $CD4^+$ T cells. Although IL-21 activates mainly STAT3, it also mediates STAT1 activation to a lesser extent that is augmented in the absence of STAT3 protein. IL-21–activated STAT1 augments *Tbx21* and *Ifng* expression, which promotes T helper type 1 (Th1) differentiation that is negatively regulated by STAT3 under the physiological condition [28]. Correspondingly, IL-21–mediated STAT1 activation and *IFNG* and *TBX21* expression was augmented in $CD4^+$ T cells from patients with autosomal dominant hyper-IgE syndrome [28], a disease caused by *STAT3* deficiency [29,30]. A potential explanation of these findings is that specific transcription factors interacts with different members of STAT proteins providing an additional layer for fine-tuning STATs transcriptional outcome.

Although Tyr^{701} phosphorylation is a key step in STAT1 activation and function, many reports have shown that STAT1 function extends beyond this phosphorylation. The first report in this perspective came from the analysis of tumor necrosis factor (TNF)-induced apoptosis [31]. By using STAT1-null human fibrosarcoma cells (U3A), Stark and colleagues have shown that U3A cells were resistant to TNF-induced apoptosis, while parental 2fTGH cells and reconstituted U3A with wild-type STAT1 (U3A-R) are sensitive. Furthermore, U3A cells showed defective expression of caspases 1,2, and 3 compared to 2fTGH and U3A-R cells. TNF-mediated apoptosis doesn't require Tyr^{701} STAT1 phosphorylation as shown by the sensitivity to apoptosis of U3A cells reconstituted with the Tyr^{701} non-phosphomimic (Y701F) STAT1 mutant (U3A-701) [31]. Also, Tyr^{701}–unphosphorylated STAT1 has been shown to regulate the expression of the gene encoding the low molecular mass polypeptide (LMP2), a component of 20S proteasome [32]. Comparison of the transcription profiles using DNA microarrays from U3A, U3A-701, U3A-R and 2fTGH showed that Tyr^{701} phosphorylation is dispensable for the consecutive expression of multiple genes such as major histocompatibility complex (MHC) class I and β2-microglobulin [33-35]. For example, in cardiac myocytes, STAT1–mediated cell death following ischemia-reperfusion injury depends on Ser^{727} but not Tyr^{701} phosphorylation [36]. STAT1 Ser^{727} phosphorylation is mediated by various kinases [37-40]. In response to IFNs, STAT1 Ser^{727} phosphorylation occurs to the Tyr^{701}-phosphorylated DNA-bound STAT1 dimers and modulates its transcriptional activity [41,42]. On the other hand, Ser^{727} phosphorylation occurs independent of Tyr^{701} phosphorylation in response to various stimuli [43,44]. In addition, upon IFN-β stimulation, IκB kinase (IKK)-related kinase ε (IKKε) mediates the phosphorylation of STAT1 at Ser^{708}, which alters the DNA binding specificity of the ISGF3 and promotes the transcription of distinct set of the ISGs [45]. Furthermore, unphosphorylated STAT1 (U-STAT1) provides additional layer of complexity to the STAT1 functions in interferon and cytokines signaling. Following IFN stimulation, Tyr^{701} phosphorylated STAT1 trans-locates to the nucleus, which is then dephosphorylated and nuclear unphosphorylated STAT1 associates with unphosphorylated STAT2 and IRF9 sustaining the expression of multiple ISGs [46,47]. Moreover, STAT1 dimerization and nuclear translocation have been reported to occur in the absence of Tyr^{701} phosphorylation and is important for regulation of distinct sets of targeted gene [48-53]. These studies show that STAT1 transcriptional outcome depends strongly on its phosphorylation status and its associating proteins.

While type I IFN-JAK–mediated Tyr^{701} STAT1 phosphorylation plays a central role in the antiviral immune response and host defense against intra-cellular bacteria like *Listeria monocytogenes*, its contribution to host defense against extracellular bacterial pathogens is elusive, with mounting evidence indicating a detrimental effect [54]. Toll-like receptor 4 (TLR4) is the mammalian receptor for bacterial lipopolysaccharide (LPS), which is major component of the outer membrane of Gram-negative bacteria [55]. While endocytosis of LPS-bound

TLR4 is essential for IFN-β production [56], the IFN-β–JAK–STAT1-Tyr^{701} phosphorylation signaling pathway is unlikely to contribute to proinflammatory cytokine production as shown by deficiencies of IFN-β, IFNAR, or TYK2 did not affect the production of proinflammatory cytokines, such as IL-6, in response to bacterial infections [57-59]. In contrast, STAT1 deficiency results in diminished production of IL-6 and enhanced survival in response to bacterial infections [60,61], indicating that the role of STAT1 extends beyond that of its Tyr^{701} phosphorylation in the context of host defense against bacterial pathogens. Supporting this premise, our work revealed that in LPS-stimulated human macrophages, STAT1 independent of its Tyr^{701} phosphorylation promoted the expression of AT-rich interactive domain-containing protein 5a (ARID5A), a post-transcriptional stabilizer of *IL6* mRNA. We found that deficiency of *IFNAR2* in LPS-stimulated human macrophages did not affect ARID5A transcripts and protein. By contrast, knocking down STAT1 in *IFNAR2* deficient human macrophages that do not express Tyr^{701} phosphorylation reduced the amounts of ARID5A transcripts and protein [62]. Our work unraveled a noncanonical IKKβ-mediated STAT1 phosphorylation at Thr^{749} downstream of TLR4 endocytosis, which promotes *ARID5A* and *IL12B* transcription. Additionally, we found that the canonical phosphorylation of STAT1 at Tyr^{701} and Ser^{727} were dispensable for the IKKβ-mediated phosphorylation of STAT1 at Thr^{749}, indicating possible distinct gene-regulatory functions of this noncanonical phosphorylation. Of note, phosphorylation of Thr^{749} did not affect STAT1 nuclear translocation. Instead, it facilitated STAT1 binding to and activation of the *ARID5A* and *IL12B* promoters. While Thr^{749} non-phosphomimic (T749A) STAT1 mutant retained its transcriptional activity on *IRF1* (containing GAS) and *MX2* (containing ISRE) promoters, it failed to induce *ARID5A* and *IL12B* promoter activity. By using site-directed mutagenesis, we found that the phosphorylation of Thr^{749} facilitates STAT1 binding to a noncanonical DNA motif (5'-TTTGANNC-3') at the promoter regions of *ARID5A* and *IL12B* conferring STAT1 with a proinflammatory function, which expands its role into regulating *IL12B* and *IL6* transcription and mRNA stabilization, respectively [62]. Collectively, our study highlights the importance of differential phosphorylation of STAT1 on its DNA binding specificity and transcriptional outcome. Thus, a better understanding of STAT1 phosphorylation status and its interaction with other transcriptional proteins may help to fine-tune therapeutic and diagnostic modalities targeting STAT1.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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