JAK-STAT 2:3, e24275; July/August/September 2013; © 2013 Landes Bioscience

CDK8 as the STAT1 serine 727 kinase?

Julia Staab, Christoph Herrmann-Lingen, and Thomas Meyer*
Department of Psychosomatic Medicine and Psychotherapy; University of Göttingen; Göttingen, Germany

Whereas cytokine-induced tyrosine phosphorylation of STAT (signal transducer and activator of transcription) proteins by JAK kinases has been well studied, much less is known about STAT-specific serine kinases and their signal-dependent regulation. The paper by Joanna Bancerek and colleagues published recently in *Immunity* reports that upon interferon-γ (IFNγ) stimulation of cells the chromatin-associated cyclin-dependent kinase 8 (CDK8) phosphorylates the regulatory serine residue 727 in the transactivation domain of STAT1. The authors state that the CDK8 module of the Mediator complex is a key component in the STAT1 signal pathway, linking serine phosphorylation to gene-specific transcriptional events.

Keywords: interferon signaling, kinase activity, serine phosphorylation, gene expression, transcriptional regulation

Abbreviations: CDK8, cyclin-dependent kinase 8; CHIP, chromatin immunoprecipitation; IFNγ, interferon-γ; RNAPII, RNA polymerase II; TAD, transactivation domain; TSS, transcription start site

Submitted: 02/28/13

Revised: 03/11/13

Accepted: 03/12/13

Citation: Staab J, Herrmann-Lingen C, Meyer T. CDK8 as the STAT1 serine 727 kinase? JAK-STAT 2013; 2: e24275; http://dx.doi.org/10.4161/jkst.24275
*Correspondence to: Thomas Meyer;
Email: thomas.meyer@med.uni-goettingen.de

Commentary to: Bancerek J, Poss ZC, Steinparzer I, Sedlyarov V, Pfaffenwimmer T, Mikulic I, et al. CDK8 Kinase Phosphorylates Transcription Factor STAT1 to Selectively Regulate the Interferon Response. Immunity 2013; 38:250-62; PMID:23352233; http://dx.doi.org/10.1016/j.immuni.2012.10.017

Although it has been well established that receptor-associated Janus kinases phosphorylate members of the STAT protein family on a critical tyrosine residue in their carboxy-termini, the kinase concomitantly phosphorylating serine residues in the transactivation domain (TAD) of STAT proteins upon exposure of cells to cytokines has long been elusive. Historically, soon after the discovery of STAT1 it was revealed that in IFNγ-treated cells phosphorylation of tyrosine 701 is an essential prerequisite for dimerization of STAT1, which requires the activity of two Janus family protein kinases, JAK1 and JAK2.[1-3] At that time in the mid-1990s, there was also evidence of a second phosphorylation event in STAT1 and STAT3 which was revealed by isolating [^{32}P]-labeled proteins from cytokine-stimulated cells and subjecting them to phospho-amino acid analysis.[4-6] The cytokine-induced increase in phospho-serine was mapped to residue 727 in the TADs of both STAT1 and STAT3.[7] Sequence alignment showed that a PMSP motif in the carboxy-termini of STAT1, STAT3, and STAT4, or the equivalent PSP motif in the homologous position in STAT5A and STAT5B, was conserved in the otherwise divergent TADs found in vertebrates.[8] By generating antibodies specifically reacting with the phosphorylated serine epitope in the C-terminal P(M)SP motifs, the presence of additional serine phosphorylation sites in STAT4, STAT5A, and STAT5B was confirmed. Using these antibodies as tools for immunodetection, a significant increase in serine phosphorylation was observed upon stimulation of cells with appropriate cytokines, which, however, was abrogated in the respective point mutants with a substitution of alanine for serine.[9-13]

Most studies have shown that post-translational modification of serine 727 is required for full transcriptional activity on cytokine-regulated genes, and there is one report on the phenotype of a STAT1 knockin-mouse line which expresses the respective serine 727 to alanine mutation.[14] STAT1-S727A mice showed a significantly reduced expression of IFNγ-induced genes and increased mortality upon infection with *Listeria monocytogenes*. Clearance of the bacteria from spleen and liver was impaired in transgenic mice lacking serine 727 phosphorylation as compared with their wild-type littermates. Given the significance of TAD phosphorylation for macrophage activation and IFNγ-dependent immune responses in vivo, the identification of the responsible kinase and its precise regulation should deepen our understanding of the design structure of the STAT1 pathway.

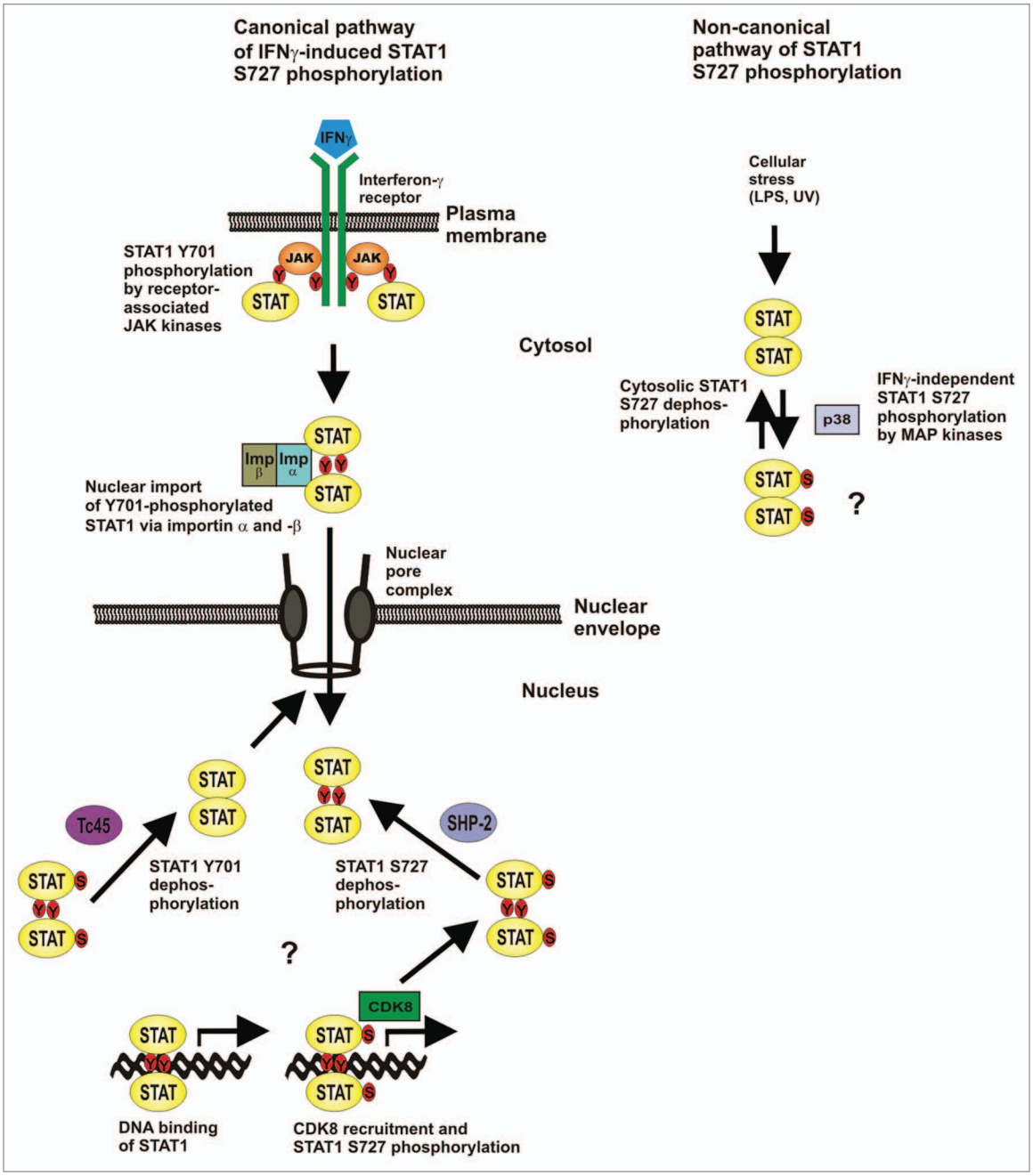

Figure 1. Canonical and non-canonical pathways leading to serine 727 phosphorylation of the transcription factor STAT1. The model proposed by Bancerek et al. depicts the role of cyclin-dependent kinase 8 (CDK8) as the nuclear STAT1 serine 727 kinase in the context of interferon-γ signaling, which includes its signal-dependent recruitment to the promoter of cytokine-driven target genes and subsequent phosphorylation of the STAT1 transactivation domain. Not shown in this model are the spatial reorganization between different dimer conformations and the nucleocytoplasmic translocation of unphosphorylated STAT1.

Previous work from the Kovarik laboratory revealed that in IFNγ-stimulated cells tyrosine 701 phosphorylation and nuclear accumulation of STAT1 are both required for TAD modification and, moreover, STAT1 needs to be assembled into chromatin-bound transcriptional complexes to become phosphorylated on serine 727 and fully biologically active.[15] Unlike the stable chromatin association required for IFNγ-induced, canonical TAD phosphorylation, STAT1 can be phosphorylated on serine 727 also independently of cytokine stimulation under conditions of cellular stress (**Fig. 1**).[16,17] Kinases which cause non-canonical TAD phosphorylation of STAT1 in response to bacterial lipopolysaccharide (LPS), UV irradiation, or tumor necrosis factor α (TNFα) include p38 mitogen-activated protein kinase (MAPK). Interestingly, Stephanou and colleagues demonstrated that serine 727 phosphorylation resulted in the enhancement of ischemia/reperfusion-induced

apoptosis in cardiac myocytes, whereas inhibition of the p38 pathway prevented TAD phosphorylation and reduced the level of cardiomyocyte cell death.[18] Data from the same laboratory also showed that the isolated carboxy-terminus of STAT1 is both necessary and sufficient to enhance sensitivity to stress-induced cell death.[19] This important finding suggests that the STAT1 TAD functions as an adaptor or co-activator for enhancing apoptosis rather than being directly involved as a signal-dependent transcription factor in modulating pro- and anti-apoptotic genes.

In the light of these observations, the identification of a STAT1-specific serine 727 kinase that selectively regulates interferon responses would add a new level of complexity to the understanding of STAT1 gene expression. In their paper, Bancerek et al. stated that CDK8 recruitment to STAT1-mediated target genes is dependent on IFNγ stimulation of cells, while the S727A mutation significantly reduced the amount of promoter-bound CDK8. From microarray analyses, the authors reported that CDK8-mediated serine phosphorylation both positively and negatively regulated the expression rate of IFNγ-induced target genes. However, they found no direct correlation between the effects of the TAD modification and gene regulation by the presence of specific STAT DNA-binding elements, such as IFNγ-activated sites (GASs) or interferon-stimulated response elements (ISREs).

When the authors examined the recruitment of RNA polymerase II (RNAPII) to transcription start sites (TSS) in IFNγ-regulated genes by means of chromatin immunoprecipitation (CHIP) assays, they observed gene-specific effects of TAD phosphorylation on the association of RNAPII to chromatin. While there was no difference between the recruitment of RNAPII to the *Irf1* TSS, the occupancy of RNAPII at the *Tap1* and *Gbp2* TSSs was significantly lower in cells expressing STAT1-S727A than the wild-type protein. These CHIP results on the chromatin recruitment of RNAPII were in agreement with their gene expression data from microarray analyses and RT-PCR validation. Moreover, the authors showed that silencing of *Ccnc*, which encodes the regulatory CycC subunit of CDK8, resulted in increased sensitivity of cells to infection with vesicular stomatitis virus, suggesting that CDK8 is indeed either an important component in the cellular protection against viral infection or, alternatively, functions as a broader, STAT1-independent regulator. The molecular mechanisms behind the assumed gene-specific divergent regulation by CDK8-mediated serine phosphorylation remain completely unclear.

The Vinkemeier laboratory uncovered the fact that accelerated nuclear export accounted for the increased transcriptional activity of serine 727-phosphorylated STAT1, shown by a prolonged phase of IFNγ-induced nuclear accumulation in the case of the S727A mutant as compared with the wild-type.[20] In view of this important finding, further research is now required to explore more fully whether or not CDK8 plays a role in marking STAT1 molecules for their subsequent dephosphorylation by the nuclear Tc45 phosphatase as a prerequisite for nuclear exit. Although there is no evidence that CDK8 acts directly in non-canonical STAT1 signaling cascades so far, the possibility remains that it may have an impact on stress-induced stimuli when the cells are pretreated with IFNγ. It is conceivable that silencing of *Ccnc* may modulate the rate of cell survival in virus-infected cells simply by enhancing apoptotic cell death in the presence of increasing concentrations of IFNγ. Thus, it will be interesting to established whether CDK8 indeed selectively controls interferon responses or may be regarded as a more general regulator, which integrates different signal inputs by sensitizing both cytokine-dependent and -independent stimuli. Thus, further research is now required, first, to confirm these data and, second, to evaluate in more detail the potential interaction between canonical and non-canonical STAT1-mediated signal pathways.

In conclusion, Bancerek et al. state that CDK8 has an impact on the activation of the majority of IFNγ-stimulated genes, suggesting a gene-specific role of this chromatin-associated kinase in STAT1-mediated transcriptional regulation. This novel finding highlights the importance of examining not only the contribution of a single key player to posttranslational modification, but also the complex interplay between the general transcription machinery and other regulatory molecules within the STAT1-specific signal pathway. Understanding the complexity, both at a gene-specific and a more global level, is what ultimately provides mechanistic insight into how antiviral responses are orchestrated in tissues and organisms.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Schindler C, Shuai K, Prezioso VR, Darnell JE Jr. Interferon-dependent tyrosine phosphorylation of a latent cytoplasmic transcription factor. Science 1992; 257:809-13; PMID:1496401; http://dx.doi.org/10.1126/science.1496401
2. Shuai K, Stark GR, Kerr IM, Darnell JE Jr. A single phosphotyrosine residue of Stat91 required for gene activation by interferon-gamma. Science 1993; 261:1744-6; PMID:7690989; http://dx.doi.org/10.1126/science.7690989
3. Shuai K, Horvath CM, Huang LH, Qureshi SA, Cowburn D, Darnell JE Jr. Interferon activation of the transcription factor Stat91 involves dimerization through SH2-phosphotyrosyl peptide interactions. Cell 1994; 76:821-8; PMID:7510216; http://dx.doi.org/10.1016/0092-8674(94)90357-3
4. Eilers A, Georgellis D, Klose B, Schindler C, Ziemiecki A, Harpur AG, et al. Differentiation-regulated serine phosphorylation of STAT1 promotes GAF activation in macrophages. Mol Cell Biol 1995; 15:3579-86; PMID:7791765.
5. Wen Z, Zhong Z, Darnell JE Jr. Maximal activation of transcription by Stat1 and Stat3 requires both tyrosine and serine phosphorylation. Cell 1995; 82:241-50; PMID:7543024; http://dx.doi.org/10.1016/0092-8674(95)90311-9
6. Zhang X, Blenis J, Li HC, Schindler C, Chen-Kiang S. Requirement of serine phosphorylation for formation of STAT-promoter complexes. Science 1995; 267:1990-4; PMID:7701321; http://dx.doi.org/10.1126/science.7701321
7. Wen Z, Darnell JE Jr. Mapping of Stat3 serine phosphorylation to a single residue (727) and evidence that serine phosphorylation has no influence on DNA binding of Stat1 and Stat3. Nucleic Acids Res 1997; 25:2062-7; PMID:9153303; http://dx.doi.org/10.1093/nar/25.11.2062
8. Decker T, Kovarik P. Serine phosphorylation of STATs. Oncogene 2000; 19:2628-37; PMID:10851062; http://dx.doi.org/10.1038/sj.onc.1203481
9. Frank DA, Mahajan S, Ritz J. B lymphocytes from patients with chronic lymphocytic leukemia contain signal transducer and activator of transcription (STAT) 1 and STAT3 constitutively phosphorylated on serine residues. J Clin Invest 1997; 100:3140-8; PMID:9399961; http://dx.doi.org/10.1172/JCI119869
10. Ng J, Cantrell D. STAT3 is a serine kinase target in T lymphocytes. Interleukin 2 and T cell antigen receptor signals converge upon serine 727. J Biol Chem 1997; 272:24542-9; PMID:9305919; http://dx.doi.org/10.1074/jbc.272.39.24542

11. Kovarik P, Stoiber D, Novy M, Decker T. Stat1 combines signals derived from IFN-γ and LPS receptors during macrophage activation. EMBO J 1998; 17:3660-8; PMID:9649436; http://dx.doi.org/10.1093/emboj/17.13.3660
12. Yamashita H, Xu J, Erwin RA, Farrar WL, Kirken RA, Rui H. Differential control of the phosphorylation state of proline-juxtaposed serine residues Ser725 of Stat5a and Ser730 of Stat5b in prolactin-sensitive cells. J Biol Chem 1998; 273:30218-24; PMID:9804779; http://dx.doi.org/10.1074/jbc.273.46.30218
13. Visconti R, Gadina M, Chiariello M, Chen EH, Stancato LF, Gutkind JS, et al. Importance of the MKK6/p38 pathway for interleukin-12-induced STAT4 serine phosphorylation and transcriptional activity. Blood 2000; 96:1844-52; PMID:10961885.
14. Varinou L, Ramsauer K, Karaghiosoff M, Kolbe T, Pfeffer K, Müller M, et al. Phosphorylation of the Stat1 transactivation domain is required for full-fledged IFN-gamma-dependent innate immunity. Immunity 2003; 19:793-802; PMID:14670297; http://dx.doi.org/10.1016/S1074-7613(03)00322-4
15. Sadzak I, Schiff M, Gattermeier I, Glinitzer R, Sauer I, Saalmüller A, et al. Recruitment of Stat1 to chromatin is required for interferon-induced serine phosphorylation of Stat1 transactivation domain. Proc Natl Acad Sci U S A 2008; 105:8944-9; PMID:18574148; http://dx.doi.org/10.1073/pnas.0801794105
16. Kovarik P, Stoiber D, Eyers PA, Menghini R, Neininger A, Gaestel M, et al. Stress-induced phosphorylation of STAT1 at Ser727 requires p38 mitogen-activated protein kinase whereas IFN-γ uses a different signaling pathway. Proc Natl Acad Sci U S A 1999; 96:13956-61; PMID:10570180; http://dx.doi.org/10.1073/pnas.96.24.13956
17. Uddin S, Majchrzak B, Woodson J, Arunkumar P, Alsayed Y, Pine R, et al. Activation of the p38 mitogen-activated protein kinase by type I interferons. J Biol Chem 1999; 274:30127-31; PMID:10514501; http://dx.doi.org/10.1074/jbc.274.42.30127
18. Stephanou A, Scarabelli TM, Brar BK, Nakanishi Y, Matsumura M, Knight RA, et al. Induction of apoptosis and Fas receptor/Fas ligand expression by ischemia/reperfusion in cardiac myocytes requires serine 727 of the STAT-1 transcription factor but not tyrosine 701. J Biol Chem 2001; 276:28340-7; PMID:11309387; http://dx.doi.org/10.1074/jbc.M101177200
19. Janjua S, Stephanou A, Latchman DS. The C-terminal activation domain of the STAT-1 transcription factor is necessary and sufficient for stress-induced apoptosis. Cell Death Differ 2002; 9:1140-6; PMID:12232802; http://dx.doi.org/10.1038/sj.cdd.4401082
20. Lödige I, Marg A, Wiesner B, Malecová B, Oelgeschläger T, Vinkemeier U. Nuclear export determines the cytokine sensitivity of STAT transcription factors. J Biol Chem 2005; 280:43087-99; PMID:16195225; http://dx.doi.org/10.1074/jbc.M509180200