Coactivator P100 Protein Enhances STAT6-Dependent Transcriptional Activation but Has No Effect on STAT1-Mediated Gene Transcription

ARTICLE *in* THE ANATOMICAL RECORD ADVANCES IN INTEGRATIVE ANATOMY AND EVOLUTIONARY BIOLOGY · JUNE 2010

Impact Factor: 1.54 · DOI: 10.1002/ar.21143 · Source: PubMed

Coactivator P100 Protein Enhances STAT6-Dependent Transcriptional Activation but Has No Effect on STAT1-Mediated Gene Transcription

XINTING WANG,[1,2,3,4] XIN LIU,[1,2,3,4] JIANFEI FANG,[1,2,3] YANXIN LU,[1,2,3] JINYAN HE,[1,2,3] XUYANG YAO,[1,2,3] ZHI YAO,[1,2,3*] AND JIE YANG[1,2,3,4]

[1]Department of Immunology, Tianjin Medical University, Tianjin, People's Republic of China
[2]Tianjin Key Laboratory of Cellular and Molecular Immunology, Tiangin Medical University, Tianjin, People's Republic of China
[3]Key Laboratory of Educational Ministry of China, Tianjin, People's Republic of China
[4]Department of Immunology, Research Center of Basic Medical Sciences, Tianjin Medical University, Tianjin, People's Republic of China

ABSTRACT

The family of STAT proteins consists of seven members that mediate highly specific functions in cytokine signaling. STAT6 is a critical regulator of transcription for interleukin-4 (IL-4)-induced genes. Activation of gene expression involves recruitment of coactivator proteins that function as bridging factors connecting sequence-specific transcription factors to the basal transcription machinery, and as chromatin-modifying enzymes. In this report, we show that the coacitivator p100 protein can interact with STAT6 through its SN domain both in vivo and in vitro, resulting in enhancement of STAT6-mediated gene transcriptional acitivation. Consistent with our previous reports, we identified intracellular localization of p100 and STAT-6 by confocal microscopy examined in response to IL-4. Moreover, in consideration of STAT molecules sharing significant homology in structure and function, we detected whether p100 can associate with STAT-1. In conclusion, this study found no evidence that p100 functions as a transcriptional coactivator for STAT1-dependent gene regulation. Anat Rec, 293:1010–1016, 2010.

Key words: p100; STAT6; STAT1; IL-4; CBP/p300; IFN-γ

Signal transducer and activator of transcription (STAT) family of transcription factors convert extracellular cytokine signals into diverse biological responses through modulation of gene transcription (Levy and Darnell, 2002). Binding of the cytokine to its receptor at the cell surface induces receptor dimerization and activation of the cytoplasmic receptor associated Janus kinases (JAK) (Kotanides and Reich, 1993). The JAK then

Grant sponsor: Ministry of Science and Technology of China (863 project); Contract grant number: 2007AA02Z115; Grant sponsor: National Natural Science Foundation of China; Contract grant numbers: 90919032, 30970562, 30670441, 30670802; Grant sponsor: Tianjin Municipal Science and Technology Commission; Contract grant numbers: 08ZCGHHZ01900, 08JCYBJC07700, 2009CB918903; Grant sponsor: 973 Program; Contract grant number: 2009CB918903; Grant sponsor: Specialized Fund for the Doctoral Program of Higher Education; Contract grant number: 20091202110001; Grant sponsor: Tianjin Educational Committee Foundation; Contract grant number: 2008ZD01.

Xinting Wang and Xin Liu contributed equally to this article.

*Correspondence to: Zhi Yao or J. Yang, Department of Immunology, Tianjin Medical University, Heping District Qixiangtai Road No.22, Tianjin 300070, People's Republic of China. Fax: +862223542581. E-mail: yangj@tijmu.edu.cn or yaozhi@tmu.cn

Received 16 September 2009; Accepted 27 December 2009

DOI 10.1002/ar.21143

Published online 11 March 2010 in Wiley InterScience (www.interscience.wiley.com).

phosphorylate a specific tyrosine residue in STAT (Darnell et al., 1994), causing STAT dimerization, translocation to the nucleus and leading to specific DNA-binding to promoter sequences. This results in transactivation of the target genes (Ihle, 1996; Leonard and Oshea, 1998). Seven mammalian STAT proteins have been identified, and they all share a similar overall structure with central DNA binding domain, Src homology 2 (SH2) domains, and the C-terminal transactivation domain (TAD), which is the most divergent part among the STATs.

STATs play prominent roles in both pro- and anti-inflammatory processes, including cell proliferation, apoptosis, and differentiation (Rawlings et al., 2004). The different STAT molecules share significant homology in structure and function. STAT6 is activated in response to IL-4 and IL-13 (Hou et al., 1994; Quelle et al., 1995). DNA-binding sites of STAT6 have been identified in the promoter regions of several IL-4- inducible genes, including CD23, major histocompatibility complex class, Ig class ε and γ1, and the 3ε-hydroxysteroid dehydrogenase (3β-HSD)type1 (Moriggl and Berchtold, 1997; Gingras et al., 1999). Transcriptional responses to IFN-γ are largely dependent on STAT1. The phosphorylated STAT1 dimers translocate to the nucleus where they bind to specific regions (gamma-activated sequence, GAS) on IFN-γ responsive promoters and modulate their transcription (Darnell et al., 1994).

p100, encoded by SND1, is a ubiquitous, multifunctional protein that can interact with and modulate a broad spectrum of proteins involved in transcription (Leverson et al., 1998; Valineva et al., 2006), for example the transcription of EBNA-2–activated gene expression (Tong et al., 1995). In addition to binding to the acidic transactivation domain of EBNA-2, p100 has been found to interact with general transcription factor TFIIE, homeodomain-containing transcription factor c-Myb. Recently, p100 has been implicated in the pathogenesis of autosomaldominant polycystic kidney disease (ADPKD) (Low et al., 2006). Notably, p100 is also a known component of the RNA-induced silencing complex (RISC), promoting cleavage of double-stranded RNA and hyperedited double stranded RNA substrates (Caudy et al., 2003). These studies suggest that p100 may have several distinct roles. Therefore, the functional understanding of the human p100 protein requires full characterization of the complex networks of protein-protein interactions. In our previous study, we discovered that p100 protein functions as a transcriptional co-activator for STAT5-dependent gene regulation and the existence of a positive regulatory loop in PRL-induced transcription (Paukku et al., 2003). Meanwhile, p100 protein was also found as a key coactivator in the STAT6 enhanceosome (Yang et al., 2002). In this regard, our study aims at investigating the potential role of p100 in STAT1-dependent gene transcription.

MATERIALS AND METHODS

Cell Culture, Transfections, and Plasmids

HeLa cells and COS-7 cells were maintained in DMEM, supplemented with 10% FBS. Transfection of HeLa cells was performed using the calcium phosphate precipitation method for luciferase assay and using lipofectamin2000 (invitrogen) for confocal microscopy. COS-7 cells were transfected by electroporation with a Bio-Rad gene pulser at 260 V/960 microfarads.

pSG5-p100-Flag, pSTAT1-HA, pSTAT6-HA, pEGFP-CI-STAT6, pERFP-p100, pERFP-p100-SN, pERFP-p100-TSN were constructed as previously described (Yang et al., 2002).

GST Pull-Down Assays

GST (glutathione S-transferase) pulldown experiments were performed as previously described (Valineva et al., 2006). GST and GST-p100-SN, GST-p100-TSN fusion proteins were produced in BL21 bacteria and purified with glutathione-Sepharose 4B beads (Amersham Biosciences, Little Chalfont, Buckinghamshire, UK) according to the manufacturer's instructions, and then incubated with total cell lysates of transfected COS7 cells. After washing, the bound proteins were eluted from beads, separated by SDS-PAGE and analyzed by silver staining or immunoblotting with mouse monoclonal anti-Flag M2 (Sigma) or mouse monoclonal anti-HA antibodies.

COS7 cells were transfected with pSTAT1-HA (hemagglutinin epitope-tagged) or pSTAT6-HA. Forty-eight hours after transfection, the total cell lysates of COS-7 cells were prepared as previously described (Valineva et al., 2006).

Co-Immunoprecipitation

COS-7 cells transfected with pSGF-p100-flag together with pSTAT6-HA or pSTAT1-HA. After 48 hr incubation, cells were collected in Nonidet P-40 lysis buffer (50 mM Tris-HCl, pH 7.6, 150 mM NaCl, 0.1mM EDTA, 0.5% Nonidet P-40, 20% glycerol, 0.1 mM sodium orthovanadate,1 mM sodium butyrate). The cell lysates were immunoprecipitated with mouse monoclonal anti-Flag M2 (Sigma) or rabbit polyclonal IgG antibody (Santa Cruz Biotechnology) as a control. The immunoprecipitated proteins were separated by SDS-PAGE and detected by blotting with anti-HA antibody.

Luciferase Assay

HeLa cells were plated in 12-well plates at a density of 3×10^4 cells per well. The cells were transfected with pSTAT6-HA plasmid, Igε-reporter luciferase construct, β-galactosidase vector, and different amount of pSG5-p100-Flag. Other cells were transfected with pSTAT1-HA plasmid, Igε-reporter luciferase construct, β-galactosidase vector, and different amount of pSG5-p100-Flag. After incubation for 24 hr, cells were starved overnight and treated or left untreated with 30 ng/mL of recombinant human IL-4 or IFN-γ for 6 hr. Cells were lysed and luciferase acitivity was measured. The luciferase values were normalized to β-galactosidase activity of the lysates.

Confocal Microscopy

HeLa cells were seeded on glass coverslips. HeLa cells were transfected with different expression plasmids as indicated in the figures by lipofectamin2000 when the cells were grown to approximately 80% confluence. After incubation for 48 hr, the cells were starved in serum-free medium for 8 hr and treated with IL-4 (100 ng/mL)

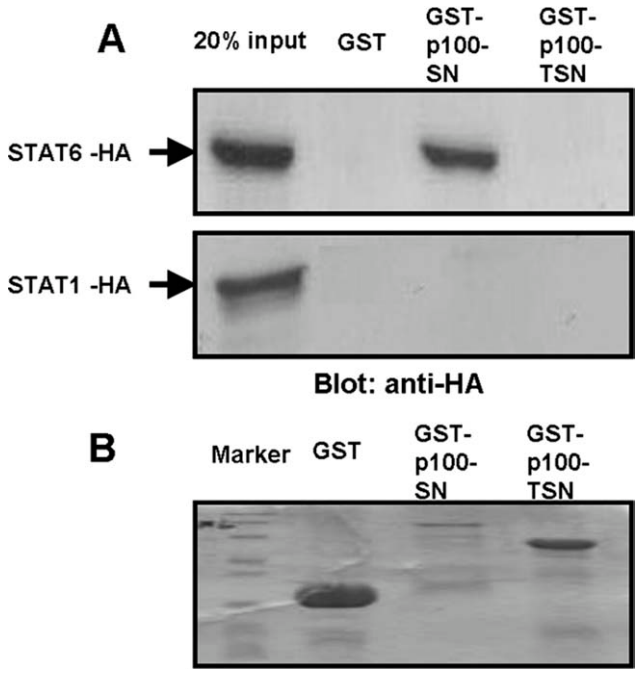

Fig. 1. Interaction domain of p100 protein and STAT6/STAT1. SN domain of p100 bind with STAT6 but not STAT1 (**A**). COS7 cells were transfected with STAT6-HA/STAT1-HA. The cell lysates were incubated with beads loaded with GST,GST-SN, or GST-TSN separately. The bound protein were subjected to SDS-PAGE and visualized by autoradiography. Twenty percentage of the in vitro lysates were included as control. **B**, Expression level of GST fusion proteins.

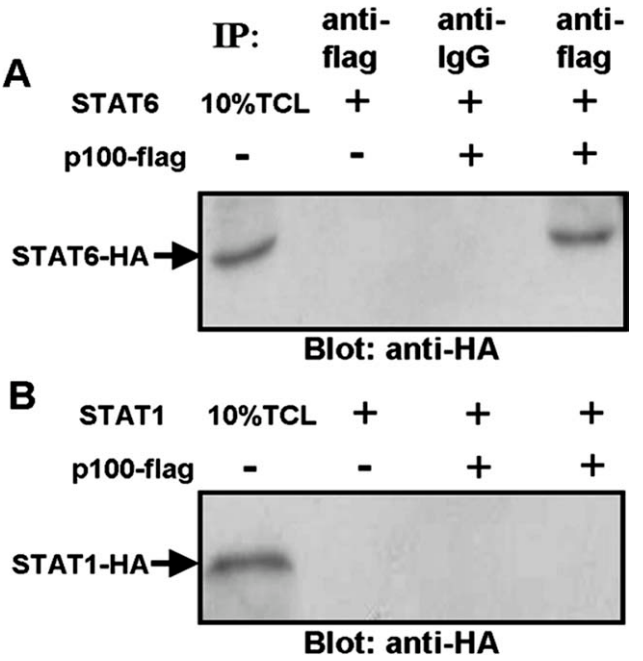

Fig. 2. STAT6/STAT1 immunoprecipitates with p100 in vivo. COS-7 cells were transfected with plasmids encoding for STAT6-HA or STAT1-HA together with p100-Flag. Cell extracts were immunoprecipitated with antiflag antibody or anti-IgG antibody as a control and immunoblotted with anti-HA antibody. Ten percentage of total cell lysate (TCL) from transfected cells was included as a control. The immunoprecipitated p100 was detected by blotting with anti-HA antibody.

for 30 min. Confocal images were collected using LSM510 program and Zeiss confocal microscope, equipped with an Argon laser (488 nm) and HeNe laser (543 nm), and a ×63 objective. Green emission was detected using a 505-nm low pass filter and red emission using a 630-nm low pass filter.

RESULTS

p100 Protein Interacts with STAT6 but Not STAT1

We performed GST pulldown assay to analyze the interaction of p100 protein with STAT1, and STAT6 as positive control. GST, GST-SN, and GST-TSN were expressed in bacteria and bound to glutathione-Sepharose beads. COS-7 cells were transfected with STAT6-HA or STAT1-HA.Then the cell lysates were incubated with beads bound GST fusion protein. As shown in Fig. 1, STAT6 interacted efficiently with p100-SN domain, but not the p100-TSN domain or GST alone. However STAT1 could not interact with p100-SN, p100-TSN, or GST alone. This result indicates that SN domain of p100 protein specifically interacts with STAT6, but neither p100-SN, nor p100-TSN, could associate with STAT1 in vitro.

As we reported earlier that p100 protein can recruit CBP to STAT6 (Valineva et al., 2005), and STAT1 could interact with CBP (Valineva et al., 2005), therefore, CBP may also act as bridge factor which recruit p100 to STAT1. To analyze whether STAT1-p100 complex formation occurs in vivo, the protein interaction between STAT1 and p100 was investigated by co-immunoprecipitation assays. COS-7 cells were cotransfected with STAT1-HA and p100-Flag. p100 was immunoprecipitated from the total cell lysates with anti-Flag antibody, and the presence of STAT1 was detected by anti-HA immunoblotting. Likewise, the same experiment was done to detect in vivo complex formation between STAT6 and p100 as positive control. As shown in Fig. 2A, STAT6 was found to coprecipitate with p100. As a control, STAT6 was not detected in anti-IgG immunoprecipitations from cells transfected with STAT6-HA and p100-Flag. However, STAT1 was not detected to bind with p100.

p100 and p100-SN Enhance STAT6-Mediated Gene Transcriptional Activation

It has been reported that CBP [cAMP response element binding protein, (CREB)-binding protein] has the capacity to facilitate the gene transcription of STAT1, STAT3, and STAT5, p100 protein was identified to interact with the HAT domain of CBP and enhance the histone acetylation of STAT6-mediated gene transcription (Valineva et al., 2005). Although we characterized that p100 could not interact with STAT1 directly, to investigate whether p100 could act as coregulator to regulate the STAT1-dependent gene transcription, we carried out luciferase reporter assay. HeLa cells were transfected with STAT1-dependent GAS luciferase reporter gene, together with increasing amounts of p100 or CBP plasmids. As a control, HeLa cells were transfected with

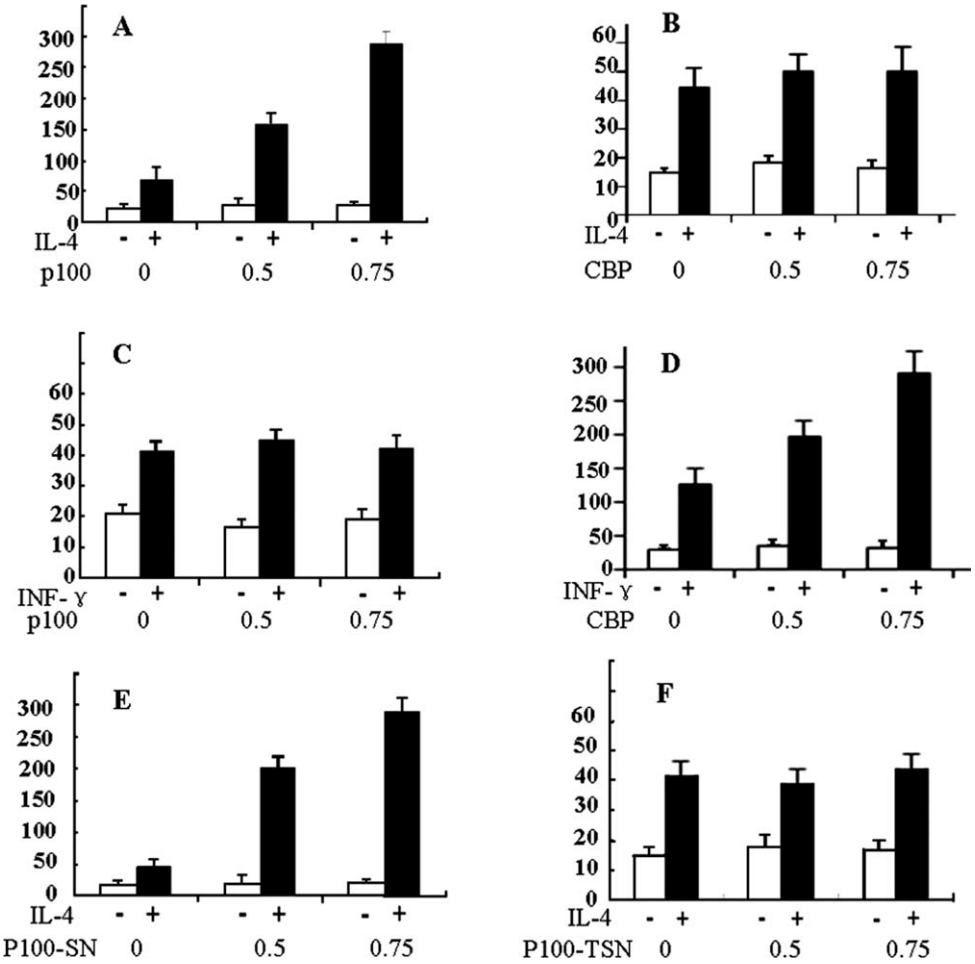

Fig. 3. p100 and p100-SN enhance STAT6-dependent transcriptional activation. HeLa cells were transfected with GAS luciferase reporter gene (0.25 μg), β-galactosidase (0.25 μg), together with increasing amounts of p100 **(C)** or CBP **(D)** plasmids. After 24 h, transfection cells were either stimulated with IFN-γ (30 ng/mL) (filled bars) or left untreated (blank bars) for 6 hr. As a control, HeLa cells were transfected with Igε-reporter (0.25 μg), β-galactosidase (0.25 μg), together with increasing amounts of p100 **(A)** or CBP **(B)** plasmids. After 24 hr, transfection cells were either stimulated with IL-4 (30 ng/mL) (filled bars) or left untreated (blank bars) for 6 hr. Cells were lysed and luciferase expression was determined. The results were normalized against β-galactosidase activity of the lysates. Likewise, p100-SN **(E)** and p100-TSN **(F)** were also detected for their transcriptional activation on STAT6 in the same manner. The RFP-p100-SN and RFP-p100-TSN plasmids were used for the reporter assay.

STAT6-dependent Igε luciferase reporter gene, together with increasing amounts of p100, or CBP plasmids.

As shown in Fig. 3, ectopic expression of p100 did not affect the basal activity of the Igε reporter, but enhanced the IL-4-stimulated reporter gene activity in a dose-dependent manner (Fig. 3A), but there is no obvious enhancement of IFN-γ-induced STAT1-mediated reporter gene activity (Fig. 3C). Interestingly, verse visa, the overexpression of CBP alone has no significantly effects on STAT6-mediated gene transcriptional activation (Fig. 3B), but dramatically enhanced the STAT1-mediated gene transcription (Fig. 3D). These data indicate that CBP may regulate the STAT1- and STAT6-mediated gene transcriptional activation via different mechanisms.

To verify the physical interaction of p100-SN domain and STAT6 in vivo, we have constructed the plasmids which express the RFP tagged p100-SN or p100-TSN fusion protein, and GFP tagged STAT6 fusion protein. To verify the proper functions of the plasmids, we performed luciferase reporter assay. HeLa cells were transfected with Igε luciferase reporter gene, with different amounts of RFP-p100-SN or RFP-p100-TSN plasmids. As shown in Fig. 3E,F, consistent with our earlier report (Yang et al., 2002), the RFP-p100-SN domain alone is sufficient to enhance the STAT6-mediated gene transcriptional activation, while RFP-p100-TSN could not. Thus, the RFP-p100-SN and RFP-p100-TSN protein has the same function as the original form.

Intracellular Localization of STAT6 and p100 Functional Domains

Our early study demonstrated that STAT6 interacts with the SN domain of p100, and SN domain is efficient to increase the IL-4/STAT6 regulated gene transcription. To further confirm the physical association of STAT6 and p100-SN domain, intracellular localization of

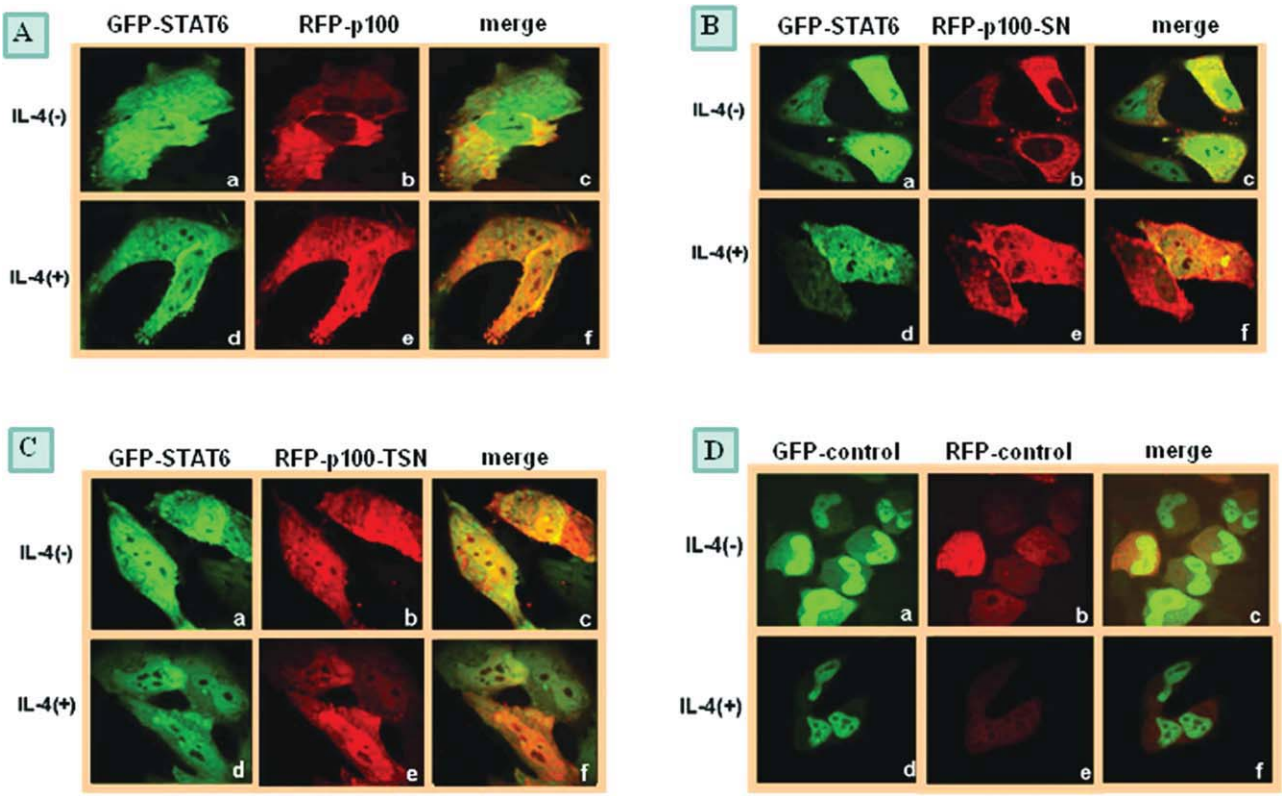

Fig. 4. Fluorescent localization of transfected STAT6,p100 and its domains. HeLa cells were starved in serum-free medium and treated with IL-4 (**a–c**) or mock-treated (**d–f**). Confocal images were collected using LSM510 program and Zeiss confocal microscopewith a ×63 objective.

overexpressed STAT6, p100, and its functional domains was investigated in HeLa cells by confocal microscopy. Cells were cotransfected with GFP-STAT6 and RFP-p100/RFP-p100-SN/RFP-p100-TSN, respectively. After overnight starvation, the cells were treated with IL-4 (100 ng/mL) for 30 min and visualized by confocal microscopy. As shown in Fig. 4, in unstimulated cells, STAT6 and p100 were distributed throughout the cells, full-length p100 protein (Fig. 4A-b) and p100-SN (Fig. 4B-b) are mainly localized in the cytoplasm, p100-TSN (Fig. 4C-b) is mainly in the nucleus. After IL-4 stimulation, more STAT6 protein translocated to the nucleus, both full-length p100 protein (Fig. 4A-e) and p100-SN (Fig. 4B-e) are also partially translocated into nucleus where also colocolized with STAT6 (Fig. 4A-f,B-f). The merged images demonstrate colocalization of STAT6 and p100/p100-SN. However, the RFP and GFP control did not show colocalization image. These results further confirmed the physical and functional interaction of STAT6 and p100-SN domain.

DISCUSSION

Transcriptional activation of eukaryotic genes is regulated by the mechanism termed combinatorial control, whereby it depends on the precise and ordered recruitment of transcriptional activators, coactivators, and general transcription factors to the promoters of target genes. The major emphasis in our laboratory is to understand the mechanism of STAT transcriptional activation. In our previous study, we identified that p100 can interact with STAT5 (Paukku et al., 2003) and STAT6 (Yang et al., 2002), and promote their transcriptional function. The different STAT proteins belong to the same family due to the similar structure and mode of action. Hereby, the aim of this study is to determine whether p100 is critical for STAT1-induced transcription. Our data show that neither p100, nor its domains can interact with STAT1, and over expression of p100 has no effect on the IFN-γ/STAT1-mediated gene transcriptional activation. Thus, it suggests that p100 is not an essential coactivator involved in STAT1 dependent transcriptional activation events. The confocal microscopy further confirmed that it is the SN domain of p100 which functionally interacts with STAT6 with IL-4 stimulation.

CBP is a multi-functional protein which plays important roles in transcriptional regulation. It acts as a protein bridge connecting sequence-specific transcription factors to the transcription apparatus, it also processes intrinsic histone acetyltransferase activity to remodel chromatin by acetylating nucleosomal histones and facilitate transcription. Therefore, recruitment of CBP may play a functional role in STATs transcriptional activation. The transactivation domain (TAD) of STATs, which is essential for gene transcription, is located in the C-terminal portion. The TAD domains are the most divergent domains in STATs, and it is reasonable to assume that STATs may utilize different coactivator

proteins to regulate gene transcription. Interestingly, all TAD of STATs is capable of recruiting CBP, though share no sequence similarity. For example, STAT1 and STAT3 interacts with N-terminus of CBP, STAT2 associates CH1 domain of CBP, STAT5 is capable of binding KIX domain of CBP/p300. Comparing with other members of the STAT family, the TAD of STAT6 is rich in porline, serine, threonine, leucine, and glutamine residues but not acidic residues. The TAD of STAT1 has been shown to interact directly with CBP/p300 (Horvai et al., 1997) while STAT6 cannot associate with CBP directly, but need p100 protein recruiting CBP to the TAD of STAT6 (Valineva et al., 2005).

From the luciferase assay, CBP alone is sufficient to promote the STAT1-mediated gene transcription, but not STAT6-mediated transcription, while ectopic expression of p100 protein alone is sufficient to enhance the STAT6-mediated transcription, but not the STAT1-mediated transcription. This is consistent with our original report that a ternary complex of STAT6, p100, and CBP is formed in cells, and that p100 can bridge the HAT activity of CBP to STAT6, while STAT1 was found to recruit CBP and HAT activity directly and p100 did not affect the recruitment of HAT activity (Valineva et al., 2005).

The function of HAT domain is to transfer acetyl group from Acetyl-CoA to acceptor substrate. There are two hypothetic mechanisms on catalyzing acetyl transfer, one is called "ping-pong" mechanism, in which the acetyl group is transferred first to an enzyme nucleophile, and then the enzyme subsequently transfers the group to an acceptor substrate. The other one is called sequential mechanism, in which the acetyl group is directly transfered to the substrate acceptor. The interaction between p100 protein and CBP may provide some insight related to facilitate the transfer of acetyl group to histones, leading further modification of chromatin structure. Our findings support a hypothesis, that p100 brings CBP to STAT6 response elements causing the nucleosomes to unfold and facilitating the access of STAT6-p100 protein complex to the basal transcription machinery and formation of the preinitiation complex. However, the precise mechanisms by which these events are connected to activation of transcription are still unknown. Furthermore, our results also indicate that various STATs are utilizing different mechanisms to recruit CBP and HAT activity. While STAT6 connects to CBP through bridging factors like p100, STAT1 was found to recruit CBP and HAT activity directly and p100 did not affect the recruitment of HAT activity.

IL-4 is a pleiotropic cytokine that regulates a variety of cellular functions, and plays an important role in the pathogenesis of asthma and allergy. In addition, IL-13 induces phosphorylation of Stat6 in lung fibroblast (Ingram et al., 2004), and many of the characteristics of airway remodeling (eosinophilia, mucous cell metaplasia, and airway fibrogenesis) are absent in the model of allergic asthma in Stat6-deficient mice (Kuperman et al., 1998). Thus, STAT6 is required for many aspects of IL-13-induced airway remodeling. Stat1 is also activated by IL-13 in a variety of lung cell types (Wang et al., 2004) and yet negatively regulates IL-13-induced signaling in pulmonary cell types (Quarcoo et al., 2004; Yu et al., 2004; Ray et al., 2005). It was demonstrated that STAT1-deficient (STAT1−/−) mice exhibited more severe pulmonary fibrosis after bleomycin injury compared with wild-type STAT1 mice (Walters et al., 2005). Therefore, STAT6 and STAT1 are both important modulators of airway remodeling but appear to exert opposing biologic effects on cytokine signaling.

It was reported that *Toxoplasma gondii* infection triggers the autonomous STAT6 activation pathway to obstruct the innate immunity induced by IFN-γ pathway (Nam, 2009). On the other hand, IFN-γ stimulation directly restricts the IL-4 action via inhibition of STAT6 phosphorylation (Huang et al., 2005) therefore may inhibit the growth and replication of *T. gondii* within the host cells. However, the underline mechanism is unknown yet.

In conclusion, we have described the mechanism by which p100 protein coactivates STAT6. We also found that p100 cannot associate with STAT1. IFN-γ only triggers the phosphorylation of STAT1 but not STAT2. However STAT2 and STAT1 form heterodimers with INF-α/β stimulation and then induce the formation of the transcription factor IFN-stimulated gene factor 3 (ISGF3) which recognize the IFN-stimulated response elements (IRSEs). Further studies are required to test whether other molecules of STAT family could interact with p100, such as STAT2. These studies might provide more insights into interaction between STAT family and p100.

ACKNOWLEDGEMENTS

The authors thank O. Silvennoinen for the plasmids and H. Zhang for technical assistance.

LITERATURE CITED

Caudy AA, Ketting RF, Hammond SM, Denli AM, Bathoorn AMP, Tops BBJ. 2003. A micrococcal nuclease homologue in RNAi effector complexes. Nature 425:411–414.

Darnell JE, Jr., Kerr IM, Stark GR. 1994. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. Science 264:1415–1421.

Gingras S, Moriggl R, Groner B, Simard J. 1999. Induction of3beta-hydroxysteroid dehydrogenase/delta5-delta4 isomerase type 1 gene transcription in human breast cancer cell lines and in normal mammary epithelial cells by interleukin-4 and interleukin-13. Mol Endocrinol 13:66–81.

Horvai AE, Xu L, Korzus E, Brard G, Kalafus D, Mullen TM, Rose DW, Rosenfeld MG, Glass CK. 1997. Nuclear integration of JAK/STAT and Ras/AP-1 signaling by CBP and p300. Proc Natl Acad Sci USA 94:1074–1079.

Hou J, Schindler U, Henzel WJ, Ho TC, Brasseur M, McKnight SL. 1994. An interleukin-4-induced transcription factor: IL-4 Stat. Science 265:1701–1706.

Huang Z, Xin J, Coleman J, Huang H. 2005. IFN-γsuppresses STAT6 phosphorylation by inhibiting its recruitment to the IL-4 receptor. J Immunol 174:1332–1337.

Ihle JN. 1996. STATs: signal transducers and activators of transcription. Cell 84:331–334.

Ingram JL, Rice AB, Geisenhoffer K, Madtes DK, Bonner JC. 2004. IL-13 and IL-1 promote lung fibroblast growth through coordinated up-regulation of PDGF-AA and PDGF-R. FASEB J 18: 1132–1134.

Paukku K, Yang J, Silvennoinen O. 2003. Tudor and nuclease-like domains containing protein p100 function as coactivators for signal transducer and activator of transcription 5. Mol Endocrinol 17:1805–1814.

Kotanides H, Reich NC. 1993. Requirement of tyrosine phosphorylation for rapid activation of a DNA binding factor by IL-4. Science 262:1265–1267.

Kuperman D, Schofield B, Wills-Karp M, Grusby MJ. 1998. Signal transducer and activator of transcription factor 6 (Stat6)-deficient mice are protected from antigen-induced airway hyperresponsiveness and mucus production. J Exp Med 187:939–948.

Leonard W, Oshea J. 1998. Jaks and STATs: biological implications. Annu Rev Immunol 16:293–322.

Leverson JD, et al. 1998. Pim-1 kinase and p100 cooperate to enhance c-Myb activity. Mol Cell 2:417–425.

Levy DE, Darnell JE, Jr. 2002. Stats: transcriptional control and biological impact. Nat Rev Mol Cell Biol 3:651–662.

Low SH, Vasanth S, Larson CH, Mukherjee S, Sharma N, Kinter MT, Kane ME, Obara T, Weimbs T. 2006. Polycystin-1, STAT6, and P100 function in a pathway that transduces ciliary mechanosensation and is activated in polycystic kidney disease. Dev Cell 10:57–69.

Moriggl R, Berchtold S. 1997. Comparison of the transactivation domains of Stat5 and Stat6 in lymphoid cells and mammary epithelial cells. Mol Cell Biol 17:3363–3678.

Nam H-W. 2009. IL-4 independent nuclear translocallization of STAT6 in HeLa cells by entry of *toxoplasma gondii*. Korean J Parasitol 47:117–124.

Quarcoo D, Weixler S, Groneberg D, Joachim R, Ahrens B, Wagner AH, Hecker M, Hamelmann E. 2004. Inhibition of signal transducer and activator of transcription 1 attentuates allergen-induced airway inflammation and hyperreactivity. J Allergy Clin Immunol 114:288–295.

Quelle FW, Schimoda K, Thierfelder W, Fischer C, Kim A, Nelms K, Paul WE, Ihle JN. 1995. Cloning of murine Stat6 and human Stat6, Stat proteins that are tyrosine phosphorylated in responses to IL-4 and IL-3 but are not required for mitogenesis Mol Cell Biol 15:3336–3343.

Rawlings JS, Rosler KM, Harrison DA. 2004. The JAK/STAT signaling pathway. J Cell Sci 117:1281–1283.

Ray R, Choi M, Zhang Z, Silverman GA, Askew D, Mukherjee AB. 2005. Uteroglobin suppresses SCCA gene expression associated with allergic asthma. J Biol Chem 280:9761–9764.

Tong X, Drapkin R, Yalamanchili R, Mosialos G, Kieff E. 1995. The Epstein-Barr virus nuclear protein 2 acidic domain forms a complex with a novel cellular coactivator that can interact with TFIIE. Mol Cell Biol 15:4735–4744.

Valineva T, Yang J, Palovuori R, Silvennoinen O. 2005. The transcriptional co-activator protein p100 recruits histone acetyltransferase activity to STAT6 and mediates interaction between the CREB-binding protein and STAT6. J Biol Chem 280:14989–14996.

Valineva T, Yang J, Silvennoinen O. 2006. Characterization of RNA helicase A as component of STAT6-dependent enhanceosome. Nucleic Acids Res 34:3938–3946.

Walters DM, Antao-Menezes A, Ingram JL, Rice AB, Nyska A, Tani Y, Kleeberger SR, Bonner JC. 2005. Susceptibility of signal transducer and activator of transcription-1-deficient mice to pulmonary fibrogenesis. Am J Pathol 167:1221–1229.

Wang I-M, Lin H, Goldman SJ, Kobayashi M. 2004. STAT-1 is activated by IL-4 and IL-13 in multiple cell types. Mol Immunol 41:873–884.

Yang J, Aittomaki S, Pesu M, Carter K, Saarinen J, Kalkkinen N, Kieff E, Silvennoinen O. 2002. Identification of p100 as a coactivator for STAT6 that bridges STAT6 with RNA polymerase II. EMBO J 21:4950–4958.

Yu C-R, Mahdi RM, Ebong S, Vistica BP, Chen J, Guo Y, Gery I, Ewuagu CE. 2004. Cell proliferation and STAT6 pathways are negatively regulated in T cells by STAT1 and suppressors of cytokine signaling. J Immunol 173:737–746.