PTB-associated Splicing Factor (PSF) Functions as a Repressor of STAT6-mediated Igε Gene Transcription by Recruitment of HDAC1*

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Regulation of transcription requires cooperation between sequence-specific transcription factors and numerous coregulatory proteins. In IL-4/IL-13 signaling several coactivators for STAT6 have been identified, but the molecular mechanisms of STAT6-mediated gene transcription are still not fully understood. Here we identified by proteomic approach that the PTB-associated splicing factor (PSF) interacts with STAT6. In intact cells the interaction was observed only after IL-4 stimulation. The IL-4-induced tyrosine phosphorylation of both STAT6 and PSF is a prerequisite for the efficient association of the two proteins. Functional analysis demonstrated that ectopic expression of PSF resulted in inhibition of STAT6-mediated transcriptional activation and mRNA expression of the Igε germline heavy chain gene, whereas knockdown of PSF increased the STAT6-mediated responses. PSF recruited histone deacetylase 1 (HDAC1) to the STAT6 transcription complex, which resulted in reduction of H3 acetylation at the promoter regions of Ig heavy chain germline Igε and inhibition of STAT6-mediated transcription. In addition, the HDACs inhibitor trichostatin A (TSA) enhanced H3 acetylation, and reverted the PSF-mediated transcriptional repression of Igε gene transcription. In summary, these results identify PSF as a repressor of STAT6-mediated transcription that functions through recruitment of HDAC to the STAT6 transcription complex, and delineates a novel regulatory mechanism of IL-4 signaling that may have implications in the pathogenesis of allergic diseases and pharmacological HDAC inhibition in lymphomas.

Signal transducers and activators of transcription (STAT) proteins are critical mediators of cytokine-induced gene expression (1). In mammalians seven STAT proteins have been identified and each STAT protein is activated by a specific cytokine and mediates its biological effects by trans-activating a unique profile of target genes (2).

Interleukin-4 (IL-4) and IL-13 are related cytokines that both activate STAT6 and have pleiotropic functions in different cells (3). In immune responses, IL-4 has important roles in Th2 functional responses such as triggering the immunoglobulin (Ig) isotype class switching and production of IgG1 and IgE (4). IL-13, on the other hand, plays important roles in airway hypersensitivity and mucus formation (5). STAT6 is a critical regulator of IL-4- and IL-13-induced gene responses, and STAT6-deficient mice fail to produce significant levels of IgE, and are protected from allergic diseases (6) and some tumors (7). Recently, constitutive activation of STAT6 has been found in several tumors (8–10), allergic diseases (11), and it predisposes toward lymphoproliferative disorder (12). Thus, understanding the mechanisms of STAT6-mediated transcription has important implications for allergic diseases and certain tumors.

Appropriate regulation of gene expression requires coordination of activating and repressing signals, which is executed by a highly integrated interplay of DNA-bound transcription factors, general transcription machinery, and coregulators that possess a variety of enzymatic activities facilitating either transcriptional activation or repression (13). In the IL-4/ STAT6 pathway, several components of the STAT6 enhanceseome have been identified and coactivator proteins such as CBP/p300, Tudor-SN, p/CIP, NcoA-1, and RNA Helicase A, have been shown to interact directly or indirectly with the transactivation domain of STAT6 (STAT6-TAD) (14–16). As an example of the regulatory network, Tudor-SN facilitates recruitment of histone acetylase activity to the human Igε promoter through formation of the STAT6-Tudor-SN-CBP ternary complex (15), and enhances STAT6-mediated transcriptional activation. These studies have shed light into the mechanisms of STAT6-mediated transcriptional activation, but the molecular mechanisms responsible for silencing the STAT6 responses at the promoter level remain unclear. In

* This work was supported by the 863 project of the Ministry of Science and Technology of China Grants 2007AA02Z115, NSFC Grants 90919032, 30970562, and 30670441, 973 program Grant 2009CB918903, Specialized Fund for the Doctoral Program of Higher Education Grant 20091121011001, Tianjin Educational Committee Foundation Grant 2008ZD01, Tianjin Municipal Science and Technology Commission Grants 08ZCHH021090 and 08JCYJC07700, Medical Research Council of Academy of Finland, Finnish Foundation for Cancer Research, Pirkanmaa Hospital District Research Foundation, Sigrid Juselius Foundation, and the Tampere Tubercolosis Foundation.

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this report, we identified the PTB-associated splicing factor (PSF)\(^4\) as a transcriptional repressor that interacts with STAT6-TAD in an IL-4-dependent manner, and inhibits STAT6-mediated Ig gene transcriptional activation by recruiting HDAC1.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Transfection**—COS-7 cells, HeLa cells, and Ramos 2G6 cells were cultured as described previously (5). COS-7 and Ramos cells were transfected by electroporation at 220 V/950 mF with a Bio-Rad gene pulser (14). The transfections of HeLa cells were performed using the calcium-phosphate co-precipitation method (14). Human IL-4 was obtained from Peprotech Inc. Trichostatin A (TSA) was obtained from Sigma.

**Plasmids**—GST-St6TAD and pcNeo-STAT6-HA were constructed as previously described (14). pcDNA3.1 His-PSF, which contains the full-length cDNA of PSF-A, was kindly provided by Dr. S. J. Lye. pGenesil-PSF-siRNA was generated by GeneSil Biotechnology Co. Ltd., China. The siRNA targeting PSF sequence contains GAGAGAGAGGAGATGTAT (sense strand), TTCAAGACG (loop), and ATCATCTTTC-CTCTCTTC (antisense strand). pGenesil-scramble siRNA (sense strand), TTCAAGACG (loop), and ATCATCTTTC-GACA (antisense strand). The luciferase reporter construct containing the STAT6 binding site from the promoter of the Ig heavy chain germline Ig gene (Ig-€-Luc) was made as described previously (14).

**GST Pulldown Assays**—GST pulldown experiments were performed as previously described (14). GST and GST-St6TAD fusion proteins were produced in BL21 bacteria and purified with glutathione-Sepharose 4B beads (Amersham Biosciences) according to the manufacturer’s instructions, and then incubated with total cell lysates of cells with/without IL-4 stimulation. After washing, the bound proteins were eluted from beads, separated by SDS-PAGE, and analyzed by silver staining or immunoblotting.

**Mass Spectrometry**—For mass spectrometric analysis, the precipitated proteins were separated by SDS-PAGE and visualized by Coomassie Blue staining. The bands corresponding to the 90-kDa proteins were cut out from the gel and subjected to trypsin digestion as described previously (14). The molecular masses of the peptide mixtures were determined by matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry. The molecular masses of the tryptic peptides were used to search the OWL protein data base for candidate proteins using the ProFound program.

**Immunoprecipitation and Antibodies**—COS-7 cells were transfected with pcNeo-STAT6-HA and pcDNA3.1 His-PSF. After 36 h, the cells were mock treated or treated with IL-4 (50 ng/ml) for 40 min, and then suspended in Nonidet P-40 lysis buffer (50 mm Tris-HCl, pH 7.6, 300 mm NaCl, 0.1 mm EDTA, 0.5% Nonidet P-40, 20% glycerol, 0.1 mm sodium orthovanadate, 1 mm sodium butyrate). The total cell lysates were incubated with mouse monoclonal anti-HA (clone 16B 12; BabCO) or anti-His (Sigma) antibody, as well as rabbit polyclonal IgG antibody (Santa Cruz Biotechnology) as a control, followed by incubation with protein G/A-Sepharose (Amersham Biosciences). The bound proteins were subjected to SDS-PAGE and blotted with anti-HA antibody or anti-His antibody.

Ramos cells were mock treated or treated with IL-4 (50 ng/ml) as indicated, and then suspended in Nonidet P-40 lysis buffer. The total cell lysates were incubated with mouse monoclonal anti-PSF (Sigma), anti-STAT6 (Upstate Biotechnology), or rabbit polyclonal IgG antibody, followed by incubation with protein G-Sepharose. The immunoprecipitated proteins were separated by SDS-PAGE and detected by blotting with anti-PSF or anti-STAT6 antibody, or anti-tyrosine-phosphorylated antibody (Zymed Laboratories Inc. Laboratories, CA).

**Reverse Transcription-PCR**—Total RNA was isolated from parental or transfected Ramos cells using TRIzol reagent (Invitrogen). One-Step RNA PCR kit (AMV; TaKaRa Biotechnology) was used for reverse transcription-PCR. PCR products were fractionated on 1.5% agarose gel and analyzed with Quantity One 4.5.6 software (Bio-Rad). The results were normalized against GADPH and presented as target mRNA to the GADPH ratio. The primer sequences were as follows: Ig heavy chain germline Ig, 5’-CACACAACATGACCCTAC-CAGC-3’ (forward) and 5’-AGTGCCAGGTGTGTTGATG-AGTC-3’ (reverse); GAPDH primers, 5’-TTGCCGACAGG-ATGCAGAAGGAAGAGATGATGAAC-3’ (forward), 5’-AGTGCCAGGTGTTGATG-AGTC-3’ (reverse).

**Chromatin Immunoprecipitation**—Formaldehyde was added to the IL-4-treated (50 ng/ml, 40 min) or mock-treated cells at a final concentration of 1%. Cross-linking was stopped by the addition of glycine to a final concentration of 100 mM. Total cell lysates were harvested with Nonidet P-40 lysis buffer. The soluble chromatin was prepared with sonication using a Vibra Cell 500 watt sonicator (Sonics & Materials, Inc., Newtown, CT). After centrifugation, samples were pre-cleared with protein G beads and immunoprecipitated with anti-STAT6, anti-acetyl-H3 (Sigma), or rabbit polyclonal IgG antibodies, and the immunocomplexes were collected onto protein G beads. Beads were washed three times with buffer containing 10 mm Tris-HCl (pH 8), 140 mm NaCl, 1 mm EDTA, 0.1% Triton X-100 and once with TE buffer (10 mm Tris-HCl, pH 8, 1 mm EDTA). The chromatin fragments were eluted from beads with elution buffer containing 62.5 mm Tris-HCl (pH 6.8), 200 mm NaCl, 2% SDS, 10 mm dithiothreitol, and cross-links were reverted by heating at 65 °C overnight. DNA was extracted with phenol/chloroform, ethanol precipitated, and analyzed for human Ig gene promoter using PCR. The PCR were performed in a 25-μl volume with 10 μl of immunoprecipitated material, using Premix Taq (TaKaRa, Japan). The extract aliquots before the immunoprecipitation step (total input chromatin) were also used for PCR analysis.

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\(^4\) The abbreviations used are: PSF, PTB-associated splicing factor; HDAC1, histone deacetylase 1; TSA, trichostatin A; STAT, signal transducers and activators of transcription; IL-4, interleukin-4; TAD, transactivation domain.
The following primers were used to detect the promoter regions of Ig heavy chain germline Igκ: 5′-TGGGCTGAGAGAGAGAGAAGA-3′ and 5′-AGCTTGCTGAGAGTTT-3′.

Luciferase Assay—HeLa cells were plated in 12-well plates at a density of 3 × 10⁴ cells per well and after 24 h transfected with pGenesil-PSF-siRNA or pCDNA3.1 His-PSF. HeLa cells were starved overnight and stimulated with 20 ng/ml of recombinant human IL-4 (PeproTech EC, London, UK) for 6 h. The cells were lysed with cell culture lysis reagent (Promega) and luciferase activity was measured as previously described (14). The luciferase values were normalized to β-galactosidase activity and are presented as the mean relative luciferase activity of three independent experiments. For all experiments, empty pSG5 vector DNA was used to balance the different amounts of DNA used in various transfections.

Immunofluorescence—HeLa cells were grown on glass coverslips. The IL-4-treated or mock-treated cells were fixed with 4% paraformaldehyde for 10 min, permeabilized with 0.5% Triton X-100 in PBS for 5 min, and blocked with 0.1% BSA in PBS for 60 min. The cells were then incubated with mouse monoclonal anti-PSF and rabbit polyclonal anti-STAT6 (Santa Cruz Biotechnology) antibodies diluted in 0.1% BSA in PBS for 2 h. After washing with PBS containing 0.1% Triton X-100 and 0.1% BSA, cells were incubated with anti-mouse Alexa Fluor 488 (Invitrogen) and anti-rabbit Texas Red (Molecular Probes, Eugene, OR) secondary antibodies in 0.1% BSA in PBS for 1 h. All incubations were performed at room temperature. Images were obtained using a ×63/1.32 oil immersion objective (Olympus, Tokyo, Japan) with an Andor iXon DV885 EMCCD camera and the Andor iQ software (Andor Technology). Images were further processed by the UltraView 4.0 software and further handled using Microsoft Photo Editor 3.01 and Adobe Photoshop 6.0 to obtain appropriate sections with best contrast/brightness and resolution.

RESULTS

Identification of PSF as a STAT6-TAD Interacting Protein—Cytokine-induced gene transcription requires precise arrangement of promoter-binding transcription factors, and hierarchical recruitment of co-regulators, which are often recruited by transactivation domains (TADs) (17). The TAD is the most divergent part of STATs. The TAD of STAT6 is rich in glutamine residues and functions as a potent independent transactivator (18, 19). To identify possible co-regulators of STAT6, the TAD of STAT6 was expressed as a glutathione S-transferase (GST) fusion protein (GST-St6TAD) and used to precipitate interacting proteins. Equal amounts of GST and GST-St6TAD fusion proteins were bound to glutathione-coupled beads (Fig. 1B) and incubated with total cell lysates of either mock or IL-4-treated HeLa cells. As shown in Fig. 1A, the GST-St6TAD fusion protein was found to interact specifically with a protein after IL-4 stimulation. The corresponding band was recovered and subjected to in-gel trypsin digestion. The molecular masses of the digested peptides were measured by MALDI-TOF mass spectrometry. The program ProFound was used to compare the mass maps obtained against theoretical tryptic peptide mass maps in the OWL protein sequence data base. The comparison identified PTB-associated splicing factor (PSF) as a STAT6-TAD interacting protein in the IL-4 stimulated but not in the mock treated lysates.

To investigate the specificity of PSF and STAT6-TAD interaction, COS-7 cells were transfected with mammalian expression plasmids encoding for His-tagged PSF (pCDNA3.1 His-PSF). After 48 h, the cells were either mock treated or stimulated with IL-4 (50 ng/ml, 40 min), and total cell lysates were collected and incubated with the GST or GST-St6TAD fusion protein-bound beads. As shown in Fig. 1C, immunoblotting results indicated that GST-St6TAD interacted with the His-PSF in the IL-4-treated lysates, but not in the mock-treated lysates. The GST pulldown assay was also performed with the endogenous protein from mock or IL-4-stimulated (50 ng/ml, 40 min) Ramos cell lysates. Analysis of the bound proteins by anti-PSF (Fig. 1D) immunoblotting demonstrated that GST-St6TAD interacted with PSF only in IL-4-stimulated cell lysates. These data demonstrate that PSF is a
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**IL-4 Stimulation Enhances Tyrosine Phosphorylation of PSF and Regulates PSF-STAT6 Association**—To investigate the mechanisms underlining the ligand-dependent association between these two proteins, we first analyzed the subcellular localization of STAT6 and PSF by immunofluorescence microscopy. As shown in Fig. 3A, in untreated HeLa cells, PSF is localized in the nucleus (a), whereas STAT6 is mainly localized in the cytoplasm but also in the nucleus to a small extent (b). The merged picture shows the distinct localization of STAT6 and PSF (c). After IL-4 stimulation (30 min), PSF remains in the nucleus (d), whereas a significant proportion of STAT6 is translocated into the nucleus (e) and co-localized with PSF in the nucleus (f). These results indicate that IL-4 stimulation induced the co-localization of STAT6 and PSF.

To further investigate the underlying mechanisms of PSF and STAT6 association, we analyzed tyrosine phosphorylation of STAT6 and PSF at different time points following IL-4 stimulation. Ramos cells were treated with IL-4 for different times as indicated, and the total cell lysates were immunoprecipitated with anti-STAT6 antibody, or rabbit anti-IgG as a control, and subjected to blotting with anti-STAT6 or anti-PSF antibody. As shown in Fig. 3B, in vivo physical complex formation between PSF and STAT6 was observed only after IL-4 stimulation. STAT6 became tyrosine phosphorylated after IL-4 stimulation, and the phosphorylation level stayed constant at different time points (Fig. 3C). PSF was tyrosine phosphorylated at the basal level even without IL-4 stimulation, and the amount of phosphorylation was gradually increased following IL-4 treatment. After 90 min treatment, the amount of phosphorylated PSF was 2-fold higher compared with the basal level (Fig. 3, D and E).

To substantiate the role of IL-4 stimulation in regulation of PSF-STAT6 association, we used COS-7 cells to carry out the co-immunoprecipitation experiments. As shown in Fig. 3F, STAT6-HA protein was ectopically expressed in COS-7 cells, which do not express detectable amounts of endogenous STAT6, and the cells were either mock treated or stimulated with IL-4. These total cell lysates were mixed and incubated with the lysates from parental COS-7 cells without (lane 3) or with IL-4 stimulation (lane 4), respectively. The mixed lysates were immunoprecipitated with anti-HA antibody and blotted with anti-PSF antibody. The results show that complex formation between STAT6 and PSF required that both proteins originate from IL-4-stimulated cells (lane 5). If only PSF originated from IL-4-stimulated cells could not efficiently form a complex with the non-treated STAT6 (lane 4). Thus, the IL-4-induced modification of both STAT6 and PSF is a prerequisite for the association of these two proteins.

**PSF Represses STAT6-mediated Gene Transcription**—To determine the functional consequences of the interaction between PSF and STAT6, HeLa cells were transfected with the Ige-Luc reporter gene, which is regulated by activated STAT6, together with increasing amounts of pcDNA-His-PSF, or pGenesil-PSF-siRNA to knockdown the PSF expression. As shown in Fig. 4A, ectopic expression of PSF did not affect the basal activity of the luciferase activity, but reduced the IL-4-stimulated reporter gene activity in a dose-dependent manner.
ner. However, knockdown of PSF enhanced the IL-4-induced reporter activity (Fig. 4B).

To verify the PSF-mediated inhibition of STAT6-dependent transcription in vivo, we analyzed mRNA expression of the Ig heavy chain germline Ig in Ramos cells by performing RT-PCR analysis. The assay conditions were adjusted to allow comparison of the Ig mRNA expression levels by normalizing to the GAPDH mRNA expression. As shown in Fig. 4C and D, ectopic expression of PSF did not affect the basal expression, but reduced the IL-4-induced expression of Ig mRNA compared with parental cells. The knockdown of PSF enhanced the IL-4-induced expression of endogenous Ig mRNA ~3-fold compared with parental cells.

To investigate whether PSF affects the binding of STAT6 to the Ig promoter, chromatin immunoprecipitation assays were performed. Ramos cells were transfected with pcDNA-His-PSF, pGenesil-PSF-siRNA, or pGenesil-scrambled-siRNA. After incubation for 48 h, the cells were stimulated with IL-4 for 40 min or left untreated. Immunoprecipitation of cross-linked chromatin was performed with anti-STAT6, or as a control with anti-rabbit IgG antibodies. DNA was extracted and analyzed for the STAT6 binding site containing the promoter regions of the Ig heavy chain gene. As shown in Fig. 5A, the protein levels of phosphorylated PSF were normalized against the protein levels of total PSF to control for variance in sample loading and transfer. The error bars represent S.D.

The COS-7 cells were transfected with STAT6-HA, and total cell lysates were collected and incubated with the total cell lysates of parental COS-7 cells treated with (lane 4) or without IL-4 stimulation (lane 3), respectively. The total cell lysates were also collected from STAT6-HA overexpression cells after IL-4 stimulation for 40 min (lane 5). After immunoprecipitation with anti-HA antibody or anti-IgG (lane 2) as negative control, the precipitated protein was separated with SDS-PAGE gel and immunoblotted with anti-HA or anti-PSF antibodies.
PSF recruits HDAC to STAT6-PSF complex to repress the gene transcription—Inhibition of histone deacetylation is correlated with transcriptional activation. PSF has been reported to recruit the mSin3A/HDAC1 complex to the promoter regions of certain genes (20, 21). We performed co-immunoprecipitation experiments to investigate whether PSF could recruit HDAC1 to STAT6. Total cell lysates from mock or IL-4-treated Ramos cells were subjected to immunoprecipitation with antibody against the PSF protein or with the control antibody. As shown in Fig. 6, HDAC1 was co-immunoprecipitated with endogenous PSF in the cell lysate (lanes 5 and 6), but was not detected in the control immunoprecipitates (lanes 3 and 4). In line with the results from the functional experiments, PSF recruited HDAC1 to STAT6 only after IL-4 stimulation (lane 6). This result indicates that IL-4 stimulation induces the recruitment of the PSF-HDAC1 complex to STAT6 in vivo.

To address the role of PSF/HDAC1 in IL-4/STAT6-induced gene expression, chromatin immunoprecipitation assays were performed to detect the acetylation of H3 at the Igε promoter region. Immunoprecipitation of cross-linked chromatin was performed with anti-acetyl-H3, or control anti-rabbit IgG antibodies and the Igε was quantitated by PCR. The result in Fig. 5B shows that overexpression of PSF reduced the H3 acetylation at the Igε promoter (lane 10) after IL-4 stimulation when compared with control cells with endogenous PSF (lane 6). To further analyze the deacetylation activity of HDAC1 in the IL-4/STAT6 pathway, Ramos cells were incubated with the HDAC inhibitor TSA. As shown in Fig. 7A, TSA treatment enhanced H3 acetylation at the Igε promoter after IL-4 stimulation (lane 8 versus 6). Importantly, the luciferase reporter experiments (Fig. 7B) support the original finding that the repressive effects of PSF on IL-4-stimulated transcription can be reverted by TSA treatment in a dose-dependent manner, whereas TSA did not alter the basal reporter activity in the absence of stimulation. Together, these data demonstrate that PSF functions as a corepressor of STAT6-mediated gene transcription via the recruitment of histone deacetylase activity.

**DISCUSSION**

Gene transcription is regulated by protein complexes that act in modulating DNA accessibility via a variety of covalent modifications of the histone tail, including acetylation, phosphorylation, methylation, and ubiquitination (22–24). Recently CoaS6 (Stat6-interacting co-factor) was reported to enhance STAT6-mediated transcription by altering the chromatin structure by modifying histones with poly(ADP-ribosylation) (25). Recently, Nigam et al. (26) have observed that
IL-4 induced transcription of the 15-lipoxygenase-1 gene requires up-regulation of acetylation of both STAT6 and H3. These findings support the concept that different enzymatic functions and their post-translational modifications coordinateantly regulate STAT6-mediated gene transcription.

Although STAT6 activation in response to IL-4 and IL-13 has been well documented, the molecular mechanisms responsible for the termination of STAT6 signaling are largely unknown. Recently, protein-tyrosine phosphatases (PTP) have been shown to dephosphorylate STAT6 and act as negative regulators of IL-4/STAT6 signaling (27). STAT6 responses are also negatively regulated by SOCS1 (28). In the present study, we characterize a novel mechanism for silencing IL-4/STAT6 gene transcription and recruitment of repressor complex PSF-HDAC1 to the STAT6-DNA promoter complex.

PSF is a multifunctional protein and has been reported to function as transcriptional repressor for several nuclear receptors including the progesterone receptor (29), androgen receptor (30), thyroid hormone and retinoic acid (31). However, the function of PSF in regulating cytokine-induced gene transcription has not been reported. In the present study, we identified PSF as an interacting protein with STAT6. Unlike most of the characterized transcription factor-coregulator interactions, the association between PSF and STAT6 was strictly IL-4 regulated. It is well established that IL-4 stimulation results in activation of tyrosine kinases JAK1 and JAK3 (32, 33) and subsequent phosphorylation of tyrosine 641 of STAT6 resulting in dimerization and nuclear translocation of STAT6, where they bind to the promoters of IL-4-responsive genes. There are a few possible mechanisms underlying the IL-4-dependent association of STAT6 and PSF. IL-4 stimulation may trigger the redistribution of STAT6 from cytoplasm to nucleus, where it could interact with the nuclear PSF. In addition, IL-4 stimulation may induce tyrosine phosphorylation of STAT6 or PSF. Although IL-4 stimulation induced the co-localization of STAT6 and PSF, it may not fully explain the ligand-dependent association, because the STAT6-TAD GST fusion protein interacts only with the PSF from IL-4-treated cell lysates. In addition, IL-4 stimulation of STAT6 was a prerequisite for association with the PSF (Fig. 3F). The GST-St6TAD is not tyrosine phosphorylated but the TAD domain alone is sufficient to activate the transcriptional activation (18, 19) and the GST-St6TAD is likely to dimerize through its GST domains and thereby mimic the phosphorylation-induced dimerization of STAT6. Thus, the dimerization or polymerization of STAT6 appears to be a prerequisite for the efficient association of PSF. On the other hand, the modification of PSF by IL-4 stimulation is also needed for the interaction, as the GST-St6TAD only interacted with the PSF from the lysate of COS-7 cells with IL-4 treatment.

Due to the ligand dependence of the interaction, we cannot distinguish between the direct or indirect association of
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STAT6 and PSF using in vitro translation assays. Therefore, we cannot exclude the possibility that another protein(s) might be involved to recruit PSF to STAT6. For example, PSF is physically associated with the carboxyl-terminal domain of RNA polymerase II (pol II) at both the initiation and elongation phases of gene transcription (34). STAT6 is also recruited to the pol II complex via the Tudor-SN protein immediately after IL-4 stimulation (14). Therefore, STAT6, RNA pol II, and PSF are likely to form the larger complex at the early stages of IL-4-induced transcription. However, the decreased binding of STAT6 to the promoter occurred at later stage, and was accompanied by increased tyrosine phosphorylation of PSF. Thus, the increased PSF tyrosine phosphorylation may mediate the recruitment and enhancement of HDAC activity and the deacetylation of histones. Further investigations are needed to clarify the underlying mechanisms.

Histone deacetylation correlates with transcriptional repression and is mediated by distinct histone deacetylase (HDAC) complexes. PSF has been reported to inhibit transcription by recruiting the mSin3A-HDAC complex to deacetylate histones during transcription elongation and modulate chromatin structure, such as IFN-γ and CIITA-induced MHC-II gene expression (20) or type II nuclear hormone receptor subfamily (21). In the present report, we demonstrate that PSF recruits HDAC1 to the STAT6 complex, and thereby, represses the STAT6-mediated IgE gene transcription, and the expression of endogenous Ig heavy chain germline IgE. This model is supported by several lines of evidence. (a) The primary function of HDAC1 is to deacetylate histones, and thereby the protein mediates transcriptional repression of genes. Consistent with this concept, we show that an increase in the amount of PSF results in reduced association of STAT6 with the IgE promoter, less acetyl-H3 at the IgE promoter, and repression of STAT6-mediated gene transcription. (b) The repressive effects can be reversed by the HDACs inhibitor TSA, which resulted in enhanced H3 acetylation at the endogenous IgE promoter, thereby reverting the suppression of STAT6-mediated gene transcription. These data demonstrate a functional link between histone deacetylase activity and the repression of STAT6-mediated gene transcription.

STAT6 requires various co-activators or co-repressors to promote and regulate transcription of endogenous genes. As STAT6 associates with both histone acetyltransferase (Tudor-SN/CBP) (15) and HDACs (PSF/HDAC1) (the present study), STAT6 seems to act as a molecular switch that dynamically associate with and regulate the function of coactivators and corepressors to regulate the higher-order chromatin remodeling of the promoter region of STAT6-bound genes, and subsequently the STAT6-mediated gene transcription. Tudor-SN recruits CBP and RNA pol II to TAD of STAT6 (15), and it also associates with p/CAF, another histone acetyltransferase to enhance histone acetylation. On the other hand, PSF acts as co-repressor to inhibit the activity through the deacetylation.

Asthma is characterized by increased STAT6 activity and IgE gene expression that are associated with an increase in histone acetylation and reduction in HDAC activities in the bronchi and alveolar macrophages (35, 36). Resistance to corticosteroids is correlated to the reduced HDAC level in some asthma patients (37). This study indicates that PSF functions as a repressor of STAT6-mediated transcription through recruitment of the HDAC complex, and delineate a novel regulatory mechanism of IL-4 signaling that may have implications in the pathogenesis of allergic diseases.

Acknowledgments—We thank S. J. Lye for PSF plasmids and H. Zhang and P. Kosonen for technical assistance.

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