A New Role for the STAT3 Inhibitor, PIAS3

A REPRESSOR OF MICROPHTHALMIA TRANSCRIPTION FACTOR*

Received for publication, September 25, 2001, and in revised form, October 29, 2001
Published, JBC Papers in Press, November 14, 2001, DOI 10.1074/jbc.M109236200

Carmit Levy‡, Hovav Nechushtan§§, and Ehud Razin¶¶
From the ‡Department of Biochemistry, Hebrew University-Hadassah Medical School, P. O. Box 12272, Jerusalem 91120, Israel and the §Department of Oncology, Hebrew University Hadassah Hospital, Ein Kerem, Jerusalem 91120, Israel

The microphthalmia transcription factor (MITF) is a basic helix-loop-helix leucine zipper (bHLH-Zip) DNA-binding protein (1). Mutation of the MITF gene, which resides at the mi locus in mice, results in deafness, bone loss, small eyes, and poorly pigmented eyes and skin (2). Mice strains harboring different mutations in the MITF gene have different phenotypes (3). One of these strains, mi/mi mice, have almost no mast cells (4). However, the transgenic tgi/tgi mice, which have an almost total lack of MITF expression (1), have a normal number of mast cells (5).

The role of MITF in gene regulation has been analyzed using two different approaches. One approach was the use of cotransfection assays and in vitro DNA binding assays, whereas the other approach utilized the available mouse models that harbor different genetic mutations in MITF. In 1996, Kitamura and his colleagues (6) demonstrated that the MITF protein in mi/mi mice is mainly located in the cytoplasm, and therefore they proposed that the presence of the mutated protein can lead to cellular defect by anchoring MITF heterodimerization partners in the cytoplasm.

MITF regulates the expression of mouse mast cell protease (mMCP)-6 (7), mMCP-5 (8) c-kit (9), p75 nerve growth factor (8), granzyme B (10), tryptophan hydroxylase (11), and cathepsin K (12). Three MCP genes are down-regulated in mast cells not only from the mi/mi strain but also from the tgi/tgi strain (11). The MCPs have distinct expression patterns in mast cells from different environments, and important physiological functions have been demonstrated for some of them (13). Thus, their down-regulation in tgi/tgi mice demonstrates that MITF plays an important role in regulation of the expression of some of the most important mast cell-specific genes. MITF also regulates the transcription of the genes that encode tyrosinase (albino locus), tyrosinase-related protein, and pink-eyed Pmel 17 (silver) (14). Since the latter three genes control pigmentation, decreased expression of MITF in melanoma patients leads to diminished pigmentation.

Like many other DNA-binding proteins, the transcription enhancing activity of MITF is influenced in a complex manner by an array of different intracellular proteins. For example, in vitro studies have indicated that MITF can form heterodimers with the four related family members TFEB, TFC, TFE3, and USF2 (15–18). MITF also synergizes with PEBP2 to increase the rate of transcription of the mMCP-6 gene (19).

Since MITF is of global importance in mast cell development, we used a yeast two-hybrid screening approach to identify some of the proteins in this effector cell that interact with MITF (20). When a mast cell library was screened with a construct that encodes the bHLH-ZIP domain of MITF, only a few genes with very high affinity to the MITF gene were isolated. Not surprisingly, one of them was TFE3, the member of the MITF-TFE family closest to MITF. Another gene found to have high affinity to MITF was the protein kinase C-interacting protein 1 (PKCI), which is a ubiquitous member of the histidine triad family of proteins (21). We accumulated clear evidence that PKCI functions as a repressor of MITF-induced transcriptional activity (20). We demonstrated that the interaction between MITF and PKCI is negatively regulated by cell surface receptor engagement (20). This suggests that PKCI functions as a transcriptional repressor by association with MITF and prevention of MITF recruitment and/or activation of target genes until the appropriate stimulus is encountered.

Using the same yeast two-hybrid screening approach in the
Suppression of MITF Transcription by PIAS3

In present work, we have identified the STAT3 inhibitor, PIAS3, as being another gene with high affinity to MITF. All seven STAT proteins directly link cytokine receptor stimulation to gene transcription by acting as both cytosolic messengers and nuclear transcription factors. In unstimulated cells, a STAT protein exists in the cytoplasm as a monomer. Upon activation by tyrosine phosphorylation in response to ligand stimulation, STATs form dimers through SH2-phosphotyrosyl interactions (22). These dimers then translocate into the nucleus to activate transcription. In a yeast two-hybrid screen aimed at the identification of potential regulators of STAT1, a protein was identified which was later named PIA1 (protein inhibitor of activated STAT1) (22). Four additional mammalian proteins related to PIA1 were identified through data base searching and cDNA library screening. One of these proteins was PIA2 (protein inhibitor of activated STAT3), which was found to be a novel protein (22). In vitro DNA binding analysis suggested that PIA3 can block the DNA binding activity of STAT3 and, in addition, that PIA3 inhibits STAT3-mediated gene activation (23).

In the present study, we present a novel function of PIA3 as a repressor of MITF-induced transcriptional activity and show that STAT3 does not interfere either in vitro or in vivo with the interaction between PIA3 and MITF.

EXPERIMENTAL PROCEDURES

Cells—Melanocytes, B16S10.9 (20), RBL, and NIH 3T3 were maintained at 37°C in growth medium, which was RPMI 1640 medium supplemented with 2 mM l-glutamine, 2 mM nonessential amino acids, 180 units/ml penicillin, 100 μg/ml streptomycin (Invitrogen), 50 μM β-mercaptoethanol (Fisher Scientific, Medford, MA), and 10% fetal calf serum.

Plasmid Construction—The bait was constructed in pAS2-1 by ligation of PCR-amplified cDNA covering the MITF cDNA fragment coding the sequence of 608–1113 (bHLH-Zip) into the BomHI and NotI sites for use in the yeast two-hybrid screening. The cDNA library was prepared from resting RBL cells mRNA by reverse transcription and cloned in the EcoRI site of the pGAD10 vector. The insert size was on average 1.7 kb and ranged from 0.3 to 3.5 kb. The cDNA encoding the open reading frame of PIA3 was subcloned into the XbaI and HindIII sites of the pGAD10 vector (Invitrogen). pGEX4T-1-PIAS3 was kindly provided by Prof. Shuai, University of California, Los Angeles, CA. PIA3 cDNA was kindly provided by Dr. Zipori, Weizmann Institute, Rehovot, Israel. The mouse MITF (1299 bp) was inserted into pGEX4T-3 vector (Amersham Biosciences, Inc.). An amplification was made from the nonmutated mouse MITF. Fidelity of all constructs was verified by direct sequencing. The luciferase reporter plasmid, pSP72, containing the MITF binding region of the promoter and the first exon of the mouse mMcP-6 gene (−191 to +26), as well as a construct with a deleted MITF binding site (−151 to +26) (7), were generously provided by Prof. Kitamura, Osaka University Medical School, Osaka, Japan.

Yeast Two-hybrid Experiments—Yeast transformation and two-hybrid screening and assays were performed according to the instructions for the MATCHMAKER two-hybrid system (CLONTECH). (20). Yeast CG1945 was transformed with pAS2-1-MITF (bHLH-Zip) to screen for proteins that were capable of interacting with MITF. We screened the pGAD10 RBL cDNA library expressed in yeast and selected the transformed yeast by growth on synthetic medium lacking Trp, Leu, and His, and containing 0.25 mM 3-aminotriazole. Five days later one of the selected colonies was regrown on the same medium. The double selected colonies were then tested for galactosidase activity. The cDNA of positive clones was rescued in Escherichia coli DH5α and subsequently sequenced.

GST Pull-down Assay—GST fusion protein MITF was expressed in protease-deficient E. coli strain B12 and purified on glutathione-Sepharose beads (Amersham Biosciences, Inc.) essentially as described before (20). Pull-down assays (24) were performed with GST-MITF fusion protein (1 μg-5 μg) bound to Sepharose beads and preincubated for 1 h at 4°C in 1 ml of binding buffer (100 mM KCl, 20 mM Hepes, 1 mM dithiothreitol, 1 mM EDTA, 5% glycerol, 0.1% Nonidet P-40). 1–10 μl of 35S-labeled PIA3 or STAT3 proteins, synthesized using the TNT-coupled rabbit reticulocyte lysate system (Promega), were added to each preincubation mix and the binding reaction carried out overnight at 4°C. Beads were washed four times in 1 ml of PBS/290 mM NaCl, boiled for 7 min in sample buffer, and aliquots were examined by electrophoresis. Integrity and quality of GST fusions were confirmed by Gelcode Bluestain Reagent, and autoradiography detected the amount of retained PIA3 or STAT3.

Transient Co-transfection Luciferase Assay—NIH 3T3 cells (5 × 105) were transfected utilizing a liposomal delivery system (Transfast, Promega) with 1 μg of reporter, 0.25 μg of pcDNA-MITF, and various concentrations of pcDNA3.1 alone as a nonspecific control or with pcDNA-MITF or pcDNA-STAT3 as the experimental variables. 105 cells/well were incubated in six-well plates for 48 h, lysed, and assayed for luciferase activity. The luciferase activity was normalized to the total protein concentration. The normalized value was then divided by the luciferase activity obtained by co-transfection of the reporter with pcDNA alone. The ratio was expressed as the relative luciferase activity.

Gel-shift Assays—Probes were double-stranded oligonucleotides of 25 bp that spanned the E-boxes of the mMcP-6 promoter as described in Ref. 8. Probes were end-labeled using DNA polymerase I large fragment (Klenow, MBI Fermentas, St. Leon-Rot, Germany). Binding reactions (20 μl) contained 100,000 cpm labeled probe, 1 μg of poly(dI-dC) (Amersham Biosciences, Inc.), 10% glycerol, 2.5 mM MgCl2, 1 mM dithiothreitol, 5% of GST-MITF, 50 μM Hepes, and 2 μg of GST-PIAS3 without increasing doses of GST-PIAS3 (2–4 μg) or GST as control. Reactions were incubated without the probe on ice for 90 min, and then the reactions were added and the reaction was incubated at room temperature for 30 min and resolved on a 6% TBE (10% Tris, 5.5% boric acid, 20 mM EDTA)-polyacrylamide nondenaturing gel (25).

Immunoprecipitation—RBL cells were lysed by the addition of 250 μl of cold lysis buffer (0.01% Triton-HCl, pH 7.4, 1% deoxycholate, 1% Triton X-100, 0.1% SDS, 0.15 mM NaCl, and 0.25 μM phenylmethylsulfonyl fluoride) and 10 μl of protease inhibitor mixture (Sigma). Cells were then homogenized and their supernatants collected after 15-min centrifugation in a microcentrifuge at 4°C. Recovered lysates were incubated with anti-mouse MITF antibody (20), anti-mouse PIAS3 antibody (Santa Cruz), or antibody against STAT3 antibody (Santa Cruz) preincubated with 10 μg of protein A-agarose (Invitrogen), and incubated with agitation overnight at 4°C. Recovered immune complexes were washed three times with lysis buffer (1:2) and then washed once with Tris-EDTA washing buffer. All buffers contained protease inhibitors. Proteins were solubilized in Laemmli sample buffer containing 0.5% SDS.

Gel Electrophoresis and Western Blots—Proteins were resolved by 10% polyacrylamide gel electrophoresis under reducing conditions and transferred to 0.45-μm nitrocellulose membranes. Visualization of reactive proteins was enhanced by enhanced chemiluminescence (20).

Indirect Fluorescent Immunocytochemistry—Cells were grown on glass coverslips in six-well plates. After extensive washing with PBS, the cells were fixed with 1.5 ml of 3.7% formaldehyde in PBS for 10 min. Fixed cells were then washed with PBS and permeabilized with 1.5 ml of Triton X-100 diluted 1:2 with PBS containing 7.5 μg of bovine serum albumin. After 45-min blocking with normal donkey serum, indirect fluorescent immunocytochemistry was carried out using rabbit anti-MITF (18) or goat anti-PIAS3 (Santa Cruz) antibodies and Cy5-conjugated donkey anti-rabbit IgG or FITC-conjugated rabbit anti-goat IgG (Jackson ImmunoResearch), respectively. Background staining was examined using the appropriate secondary antibody alone. Specimens were examined, analyzed, and photographed by laser scanning confocal microscopy using the Zeiss LSM 410 confocal laser scanning system connected to a Zeiss Axiowert 135M microscope with 63×/1.2 C-Apochromat water immersion lens (Zeiss, Jena, Germany). Green fluorescence of FITC and Cy5 labels was excited with argon laser (488 nm excitation line with 515-nm-long pass barrier filter).

RESULTS

Association of MITF and PIAS3—We used the yeast two-hybrid system to screen for MITF-interacting proteins, as described previously (20). Quiescent RBL cells were induced to produce the cDNA library that was screened, thus the proteins found to associate with MITF did not include any of those whose mRNA expression is induced in activated cells. One of the isolated cDNA clones encoded the PKC1 protein, whereas another isolated cDNA encoded the PIAS3 protein.

The direct association between full-length MITF and PIAS3 was determined using an in vitro pull-down assay. MITF was
expressed in bacteria as GST fusion, immobilized on glutathione-agarose beads, and assayed for its ability to retain in vitro translated PIAS3 labeled with [35S]methionine. PIAS3 bound to GST-MITF but not to control GST alone. Thus, PIAS3 and full-length MITF can complex with each other both in yeast and in vitro (Fig. 1).

To obtain evidence of the possible existence in vivo of an MITF-PIAS3 complex, we then investigated whether immunoprecipitation of MITF from quiescent RBL cells or melanocytes would result in specific co-immunoprecipitation of PIAS3. This was carried out by incubating the RBL lysates with either anti-MITF or anti-PIAS3 antibody prebound to protein A-agarose beads. The recovered and resolved immune complexes showed the co-immunoprecipitation of PIAS3 with MITF using either immunoprecipitation approach (Fig. 2). Thus, PIAS3 forms a complex with MITF in mast cells and melanocytes.

Next we determined whether MITF could form a complex with PIAS3 and STAT3 in vivo. First we examined whether in vitro translated STAT3 could associate directly with GST-MITF fusion protein immobilized on glutathione-agarose beads. The results presented in Fig. 3 clearly show that STAT3 does not bind to MITF. Furthermore, in another set of in vitro pull-down assays, we demonstrated that STAT3 could not bind to the PIAS3-MITF complex (Fig. 4). Immunoprecipitation of MITF from quiescent RBL cells resulted in specific co-immunoprecipitation of PIAS3, but not STAT3. Thus it seems that, at least in quiescent cells, a complex containing MITF, PIAS3, and STAT3 does not exist.

The gel-shift assay was performed to determine whether PIAS3 can modulate the binding of MITF to E-boxes in the mMCP-6 promoter (7) using GST-MITF and GST-PIAS3 fusion proteins (Fig. 5). A marked decrease in MITF binding to the oligonucleotides was observed when the GST-MITF was preincubated with increasing doses of GST-PIAS3. Neither GST-PIAS3 nor GST alone showed any binding to this oligonucleotide. These results suggest that PIAS3, by its association with MITF, decreases MITF’s binding to DNA.

Regulation of MITF Transcriptional Activity by PIAS3—To test the effect of PIAS3 interaction on MITF transcriptional activity, we chose to work with cells that did not abundantly express endogenous MITF. We have previously shown that MITF is not present in NIH 3T3 fibroblasts (20). NIH 3T3 fibroblasts were co-transfected with a luciferase reporter plasmid containing the mouse mMCP-6 promoter and pcDNA constructs of either MITF alone or MITF and PIAS3. Up to 94% inhibition of the luciferase activity was observed when the NIH 3T3 fibroblasts were co-transfected with 0.25 μg of MITF and 1 μg of PIAS3 (Fig. 6). Moreover, the extent of inhibition increased with increasing concentrations of PIAS3. Co-transfection of MITF with the pcDNA vector alone showed no inhibitory effect on the MITF-induced luciferase activity.

To test the effect of STAT3 on the suppression of MITF transcriptional activity by PIAS3, NIH 3T3 fibroblasts were co-transfected with a luciferase reporter plasmid containing the pcDNA constructs of either MITF or MITF and PIAS3 and increasing concentrations of STAT3 (Fig. 7). STAT3 did not show any effect on the inhibition of MITF transcriptional activity caused by PIAS3 in quiescent cells.

Intracellular Localization of PIAS3—We have previously shown that MITF is localized in the nucleus of RBL cells (20). The intracellular localization of MITF and PIAS3 was determined by indirect fluorescent immunocytochemistry. For MITF localization, cells were labeled with Cy5-conjugated secondary antibody (red) that recognizes the FITC-conjugated primary antibody. For PIAS3 localization, the cells were labeled with Alexa Fluor 488-conjugated secondary antibody (green) that recognizes the primary antibody. For MITF localization, the cells were labeled with FITC-conjugated secondary antibody (red) that recognizes the highly specific anti-MITF antibody. C. RBL cells were stained with anti-MITF (A, C) and anti-PIAS3 (B) antibodies. One representative of three experiments is shown.

Fig. 1. PIAS3 binds MITF in vitro. [35S]-Labeled PIAS3 was incubated with GST-MITF immobilized on glutathione-Sepharose beads, and, after washing, retained [35S]-labeled PIAS3 protein was examined by SDS-PAGE and autoradiography. One representative of three experiments is shown.

Fig. 2. Association of MITF with PIAS3 in RBL cells and melanocytes. Western blot of immunoprecipitation of PIAS3 with MITF from RBL cells (A, B) and melanocytes (C). Immunoprecipitation from lysates of RBL cells and mouse melanocytes with either anti-PIAS3 (A, C) or anti-MITF (B) protein A-agarose beads. Blots were probed with either anti-MITF (A, C) or anti-PIAS3 (B) antibodies. One representative of three experiments is shown.

Fig. 3. PIAS3 but not STAT3 binds MITF in vitro. [35S]-Labeled PIAS3 or [35S]-labeled STAT3 (+, 15 ng; +, 30 ng) were incubated with either GST or GST-MITF immobilized on glutathione-Sepharose beads, and, after washing, retained [35S]-labeled PIAS3 or [35S]-labeled STAT3 protein were examined by SDS-PAGE and autoradiography.

Fig. 4. STAT3 does not bind in vitro to PIAS3-MITF complex. [35S]-Labeled PIAS3 or [35S]-labeled STAT3 (15 ng) was incubated with GST-MITF immobilized on glutathione-Sepharose beads with or without increasing concentrations of PIAS3. After washing, retained [35S]-labeled PIAS3 or [35S]-labeled STAT3 protein were examined by SDS-PAGE and autoradiography.
DISCUSSION

MITF has been shown to be capable of binding to several transcription factors, including c-Fos (26), USF2 (18), and TFE3 (which also associates with MITF in the yeast two-hybrid screening of mast cells (20)). In addition, we have described the binding of PKCI to MITF in quiescent cells (20). Now we show that PIAS3 can interact with MITF and inhibit its transcriptional activity.

Evidence supporting the idea that PIAS3 is a physiological regulator of MITF is presented in this article. PIAS3 was isolated from the yeast two-hybrid cDNA library using truncated wild-type MITF as the bait. It bound to full-length MITF in a pull-down assay in vitro. Moreover, MITF and PIAS3 were found as a complex in quiescent RBL cells and melanocytes in co-immunoprecipitation assays. Using the ability of MITF to activate the transcription of a reporter gene under the control of the MCP-6 gene, we demonstrated that PIAS3 inhibits MITF function in vivo.

The expression of mMCP-6 is highly restricted to mast cells (27), and even fibroblasts in normal tissue do not express this protease (28). mMCP-6 has been shown to be a very potent chemoattractant for neutrophils, suggesting that it plays an important function during many inflammatory processes (29). Kitamura and his group (7) have also shown that a short sequence from the MCP-6 promoter contains an MITF response element. Thus the study of MITF activity through experiments performed with the MCP-6 promoter is highly relevant for a better understanding of MITF regulation in mast cells.

PIAS3 inhibited the MCP-6 promoter controlled transcription in a dose-dependent manner. The addition of recombinant PIAS3 to MITF and the target oligonucleotide resulted in the decreased binding of MITF containing complexes to DNA and not in the appearance of slower migrating complexes containing DNA bound to MITF and cytosolic and nuclear staining with anti-MITF and cytosolic and nuclear staining with anti-PIAS3. One representative experiment of three is shown.

Although MITF has been shown to have key regulatory roles both in mast cells and in melanocytes (2), the role played by STAT3 in receptor-mediated signaling has been poorly reported in mast cells. STAT3 is a latent transcription factor that mediates cytokine- and growth factor-directed transcription. In a variety of hematopoietic-derived cells, receptor stimulation leads to phosphorylation of tyrosine residues of STAT, which rapidly triggers DNA binding and STAT-mediated gene transcription (22, 23, 30). Our data strongly suggest that STAT3 does not associate with PIAS3 that is already associated with...
MITF. Co-transfection of MITF with increasing concentrations of PIAS3 caused dose-dependent repression of MITF transcriptional activity. However, co-transfection of MITF, PIAS3, and STAT3 did not affect the inhibition of MITF transcriptional activity by PIAS3. Thus it seems that the binding of PIAS3 in vivo to MITF might occur under circumstances in which its binding to STAT3 is minimal.

The network of transcription factors involved in mast cell regulation is very complex. Here our results point toward two new participants in this network, PIAS3 and STAT3. Understanding the circumstances and mechanisms involved in the regulation of STAT3 and MITF by PIAS3 is our next research goal.

REFERENCES
1. Hodgkinson, C. A., Moore, K. J., Nakayama, A., Steingrimsson, E., Copeland, N. G., Jenkins, N. A., and Arnekeiter, H. (1993) Cell 74, 395–404
2. Hallsson, J. H., Favor, J., Hodgkinson, C., Glaser, T., Lamoreux, M. L., Magnusdottir, R., Gunnarsson, G. J., Sweet, H. O., Copeland, N. G., Jenkins, N. A., and Steingrimsson, E. (2000) Genetics 155, 291–300
3. Morii, E., Ogihara, H., Oboki, K., Kataoka, T. R., Maeyama, K., Fisher, D. E., Lamoreux, M. L., and Kitamura, Y. (2001) Blood 98, 2577–2579
4. Kitamura, Y., Morii, E., Jippo, T., and Ito, A. (2000) Int. J. Hematol. 71, 197–202
5. Kitamura, Y., Morii, E., Ogihara, H., Jippo, T., and Ito, A. (2001) Int. Arch. Allergy Immunol. 124, 16–19
6. Takebayashi, K., Chida, K., Tsukamoto, I., Morii, E., Munakata, H., Arnekeiter, H., Kuruki, T., Kitamura, Y., and Nomura, S. (1996) Mol. Cell. Biol. 16, 1203–1211
7. Morii, E., Tsujimura, T., Jippo, T., Hashimoto, K., Takebayashi, K., Tsujishita, K., Nomura, S., Yamamoto, M., and Kitamura, Y. (1996) Blood 88, 2488–2494
8. Morii, E., Jippo, T., Hashimoto, K., Kim, D.-K., Lee, Y.-M., Ogihara, H., Tsujino, K., Kim, H.-M., and Kitamura. Y. (1997) Blood 90, 3057–3066
9. Isozaki, K., Tsujimura, T., Nomura, S., Morii, E., Koshimizu, U., Nishimune, Y., and Kitamura. Y. (1994) Am. J. Pathol. 145, 827–836
10. Ito, A., Morii, E., Maeyama, K., Jippo, T., Kim, D.-K., Lee, Y.-M., Ogihara, H., Hashimoto, K., Kitamura, Y., and Nojima. H. (1998) Blood 91, 3210–3221
11. Ito, A., Morii, E., Kim, D.-K., Kataoka, T. R., Jippo, T., Maeyama, K., Nojima, H., and Kitamura. Y. (1999) Blood 93, 1189–1196
12. Matyukova, G., Weilbaecher, K. N., Horstmann, M., Rieman, D. J., Fisher, D. Z., and Fisher, D. E. (2001) Proc. Natl. Acad. Sci. U. S. A. 98, 5798–5803
13. Huang, C., Sali, A., and Stevens, R. L. (1998) J. Clin. Immunol. 18, 169–183
14. Bentley, N. J., Eisen, T., and Goding, C. R. (1994) Mol. Cell. Biol. 14, 7996–8006
15. Carr, C. S., and Sharp, P. A. (1990) Mol. Cell. Biol. 10, 4384–4388
16. Beckmann, H., Su, L. K., and Kadesh, T. (1990) Genes Dev. 4, 167–179
17. Zhao, G. Q., Zhao, Q., Zhou, X., M. G., M., and De Crombrugghe, B. (1993) Mol. Cell. Biol. 13, 4505–4512
18. Nechushtan, H., Zhang, Z. C., and Razin, E. (1997) Blood 89, 2999–3008
19. Ogihara, H., Kanno, T., Morii, E., Kim, D.-K., Lee, Y.-M., Sato, M., Kim, W.-Y., Nomura, S., Ito, Y., and Kitamura, Y. (1999) Oncogene, 18, 4632–4639
20. Razin, E., Zhang, Z. C., Nechushtan, H., Frenkel, S., Lee, Y.-N., Arushchandran, R., and Rivera, J. (1999) J. Biol. Chem. 274, 34272–34276
21. Lima, C. D., Klein, M. G., and Hendrickson, W. A. (1997) Science 278, 286–290
22. Shuai, K. (2000) Oncogene 19, 2638–2644
23. Chung, C. D., Liao, J., Liu, B., Rao, X., Jay, P., Berta, P., and Shuai, K. (1997) Science 278, 1803–1805
24. Goldstein, R. E., Jinene, G., Cook, O., Gur, D., and Paroush, Z. (1999) Development (Camb.) 126, 3747–3755
25. Lewin, I., Jacob-Hirsch, J., Zhang, Z. C., Kupershstein, V., Szallasi, Z., Rivera, J., and Razin, E. (1996) J. Biol. Chem. 271, 1534–1539
26. Sato, M., Morii, E., Takebayashi-Suzuki, K., Yasui, N., Ochi, T., Kitamura, Y., and Nomura, S. (1999) Biochem. Biophys. Res. Commun. 254, 384–387
27. Reynolds, D. S., Stevens, R. L., Lane, W. S., Carr, M. H., Austen, K. F., and Serafin, W. E. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 3230–3234
28. Stevens, R. L., Friend, D. S., McNeil, H. P., Schiller, V., Ghidey, N., and Austen, K. F. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 128–132
29. Huang, C., Friend, D. S., Qu, W. T., Wong, G. W., Morales, G., Hunt, J., and Stevens, R. L. (1998) J. Immunol. 160, 1910–1919
30. Junicho, A., Matsuda, T., Yamamoto, T., Kishi, H., Korkmaz, K., Saito, K., Fuse, H., and Muragushi, A. (2000) Biochem. Biophys. Res. Commun. 278, 9–13
A New Role for the STAT3 Inhibitor, PIAS3: A REPRESSOR OF MICROOPHTHALMIA TRANSCRIPTION FACTOR
Carmit Levy, Hovav Nechushtan and Ehud Razin

J. Biol. Chem. 2002, 277:1962-1966.
doi: 10.1074/jbc.M109236200 originally published online November 14, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M109236200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 30 references, 21 of which can be accessed free at
http://www.jbc.org/content/277/3/1962.full.html#ref-list-1