Stat3 Dimerization Regulated by Reversible Acetylation of a Single Lysine Residue

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Upon cytokine treatment, members of the signal transducers and activators of transcription (STAT) family of proteins are phosphorylated on tyrosine and serine sites within the carboxyl-terminal region in cells. We show that in response to cytokine treatment, Stat3 is also acetylated on a single lysine residue, Lys^{685}. Histone acetyltransferase p300–mediated Stat3 acetylation on Lys^{685} was reversible by type I histone deacetylase (HDAC). Use of a prostate cancer cell line (PC3) that lacks Stat3 and PC3 cells expressing wild-type Stat3 or a Stat3 mutant containing a Lys^{685}-to-Arg substitution revealed that Lys^{685} acetylation was critical for Stat3 to form stable dimers required for cytokine-stimulated DNA binding and transcriptional regulation, to enhance transcription of cell growth–related genes, and to promote cell cycle progression in response to treatment with oncostatin M.

The STAT proteins are latent self-signaling transcription factors in cytoplasm used by most cytokine receptors to rapidly turn on gene expression in nuclei. The regions of STAT that are upstream from the linker to the C terminus appear to participate in protein-protein interactions. This region includes the Src homology 2 (SH2) domain, a short segment (about 30 to 40 amino acids in length) of unknown function, and the transcriptional activation domain (TAD) required for cytokine-induced transcriptional activation. Function-related posttranslational modifications of STAT proteins in response to treatment with cytokine or growth factor include phosphorylation of a single tyrosine residue in the short segment and a single serine residue in the TAD. The SH2 domain of STAT mediates STAT docking on phosphotyrosine (pTyr) motifs of cytokine or growth factor receptors (*1*), and a role in STAT dimerization via reciprocal pTyr-SH2 domain interaction has been also proposed (*2–4*). However, unphosphorylated or tyrosine-mutated STAT proteins can still form dimers and induce transcription (*5–8*), suggesting that another type of regulation contributes to the formation of stable STAT dimers. The C-terminal region of STAT interacts with other proteins during signaling or transcription. For instance, members of the CREB-binding protein (CBP)/p300 family have intrinsic histone acetyltransferase (HAT) activity, associate with various STAT family members within both the C-terminal TAD and N-terminal domain, and increase STAT activity in transcription (*9–12*).

To explore whether STAT proteins themselves are acetylated, we examined Stat3 activation by oncostatin M (OSM), a member of the interleukin-6 (IL-6) cytokine family, and by interferon-α (IFN-α), a type I interferon. In MCF-7 breast cancer cells, OSM treatment induced Stat3 acetylation within 15 min, an event that was maintained for hours (Fig. 1A). Likewise, Stat3 was also acetylated in response to IFN-α treatment in HeLa cells (Fig. 1A). We further examined the location(s) of Stat3 acetylation by separating cytoplasmic and nuclear fractions of MCF-7 cells. OSM-mediated Stat3 phosphorylation and acetylation were detected primarily in the cytoplasmic fraction (fig. S1), consistent with detection of p300 in the cytoplasm of some cell types (*13*). To determine whether p300 might mediate Stat3 acetylation, we transfected cMyc-tagged Stat3 with hemagglutinin (HA)–tagged p300, CBP, or p300/CBP-associated factor (PCAF). In 293T cells, transient transfection of either p300 or CBP increased Stat3 acetylation appreciably, whereas PCAF transfection caused only a small increase in Stat3 acetylation (Fig. 1B).

We evaluated the effect of phosphorylation on Stat3 acetylation by comparing the response of Stat3^{Y705F}, Stat3^{S727A}, and Stat3^{R585Q} (a Stat3 mutant in which Arg^{585} in the SH2 domain was substituted with glutamine) to OSM with the response of wild-type Stat3 in PC3 cells. All three Stat3 mutants were acetylated in cells treated with OSM (*14*). Similarly, transient transfection of 293T cells with p300 caused acetylation of these Stat3 variants (*14*), suggesting that Stat3 phosphorylation and the SH2 domain activity are not prerequisites for Stat3 acetylation. Treatment of 293T cells with trichostatin A (TSA), a broad inhibitor of histone deacetylases (HDACs), further augmented Stat3 acetylation in response to p300 transfection or IFN-α treatment (Fig. 1C), suggesting a role of HDAC factors in Stat3 deacetylation.

Type I HDAC factors may regulate transcription activation by STATs (*15*). To determine whether type I HDAC factors contribute to Stat3 deacetylation, we transfected Hdac1, Hdac2, and Hdac3 with p300 into 293T cells. Stat3 acetylation mediated by p300 was attenuated by Hdac1 and Hdac2 and was almost completely blocked by Hdac3 (Fig. 1D). TSA treatment blocked the negative effect of type-I HDAC on Stat3 acetylation (Fig. 1D). In an in vitro assay, immunoprecipitated acetyl-Stat3 with immunopurified type I HDAC converted Stat3 from its acetylated form into the deacetylated form (Fig. 1E). STAT-dependent transcription can be increased by p300 or CBP transfection (*9*, *10*). To correlate acetylation or deacetylation with Stat3 transcriptional activity, we transfected cells with p300 and HDAC and measured Stat3-dependent luciferase activity. Stat3 was activated in cells transfected with an activated mutant of the tyrosine kinase RET. Stat3 activation was further enhanced in cells transfected with p300 but was inhibited if cells were transfected with type I HDACs. The effect of these HDAC factors was abolished in cells treated with TSA (Fig. 1F). Decreased expression of p300 by up to 90% by small interfering RNA (siRNA) attenuated Stat3 transcriptional activity in response to IL-6 treatment (Fig. 1G). These results indicate that p300 and type I HDAC family members that are present in most cell types may modulate cytokine-induced Stat3 acetylation and deacetylation.

In both MCF-7 and HeLa cells, we detected a complex formation between p300 and Stat3, which was stabilized by treatment with cytokine (Fig. 2A) (*9–11*). We explored Stat3 interaction with p300 and with type I HDACs and characterized the domains of Stat3 involved in these interactions. A series of domain-truncated Stat3 constructs were created and transfected with p300 or Hdac3 into 293T cells (fig. S2A). p300 interacts with Stat1 and Stat2 within both C-terminal TAD and N-terminal domain (*9*, *10*), as confirmed for Stat3 (Fig. 2B). The interaction of Stat3$^{1-585}$, which carries the linker domain, with p300 appears to be stronger than that of Stat3 domain mutants that lack the linker domain (Fig. 2B). We transfected Stat3 with individual HDAC factors in 293T cells and determined that Stat3 coimmunoprecipitated with Hdac1, Hdac2, and Hdac3 (Fig. 2C). Because Hdac3 displayed the strongest inhibitory effect on Stat3 deacetylase activity, we studied this interaction in

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more detail. Coimmunoprecipitation experiments revealed that Stat3 N-terminal region (amino acids 1 to 130) bears an Hdac3 docking site (Fig. 2D). Stat3[1-585] also showed strong interaction with Hdac3. Hdac1, Hdac2, and Hdac3 carry a homologous C-terminal region, which plays a regulatory role in HDAC catalytic activity (*16*). Stat3 immunoprecipitated with full-length or the C-terminal (amino acids 232 to 428) portion of Hdac3 but not with constructs of Hdac3 that lacked the C-terminal sequence (Fig. 2E). The interactions between endogenous Stat3 and type I HDAC factors in HeLa cells were detected by coimmunoprecipitation analysis (fig. S2B).

To identify the lysine residues of Stat3 acetylated by p300, we analyzed Stat3 truncation variants. Wild-type and truncated Stat3 constructs were transfected with p300 into 293T cells. Stat3 immunoprecipitates were prepared and analyzed with an antibody to acetylated lysine on Western blots. Stat3[1-722] lacking a C-terminal p300-docking site and wild-type Stat3 were comparably acetylated by p300. In contrast, neither Stat3[1-465] nor Stat3[1-585] was acetylated by p300 despite their interaction with p300 (Fig. 3A). Thus, the Stat3 C-terminal region from Met[586] to Met[770] appears to contain the lysine residues acetylated by p300. Stat3[465-770] contains 19 lysine residues, which are all conserved. In 293T cells, Stat3 acetylation by p300 was abrogated when a Lys[685]-to-Arg substitution was introduced (Fig. 3B). Mutation of Lys[685] to Arg in Stat3 did not cause any detectable changes in its complex formation either with p300 or with HDAC factors (*17*). In some transcription factors, lysine residues within the DNA binding domains are acetylated (*18*, *19*). We mutated each of these lysine residues identified within the Stat3 DNA binding domain and Stat3 N-terminal domain where Stat3 provides another binding site for p300. However, all such mutants were acetylated by p300 to levels comparable to those of wild-type Stat3, suggesting that it is unlikely that these lysine sites are acetylated by p300 (fig. S3A) (*17*).

Stat3 acetylation was further examined with an in vitro acetylation assay. Bacterially produced, purified, wild-type glutathione *S*-transferase (GST)–Stat3 but not GST-Stat3-K[685]R was acetylated by purified p300 or CBP (Fig. 3C). The antibody to acetylated lysine recognizes the acetylated peptide containing Stat3 Lys[685] site and flanking sequences but not the unacetylated peptide

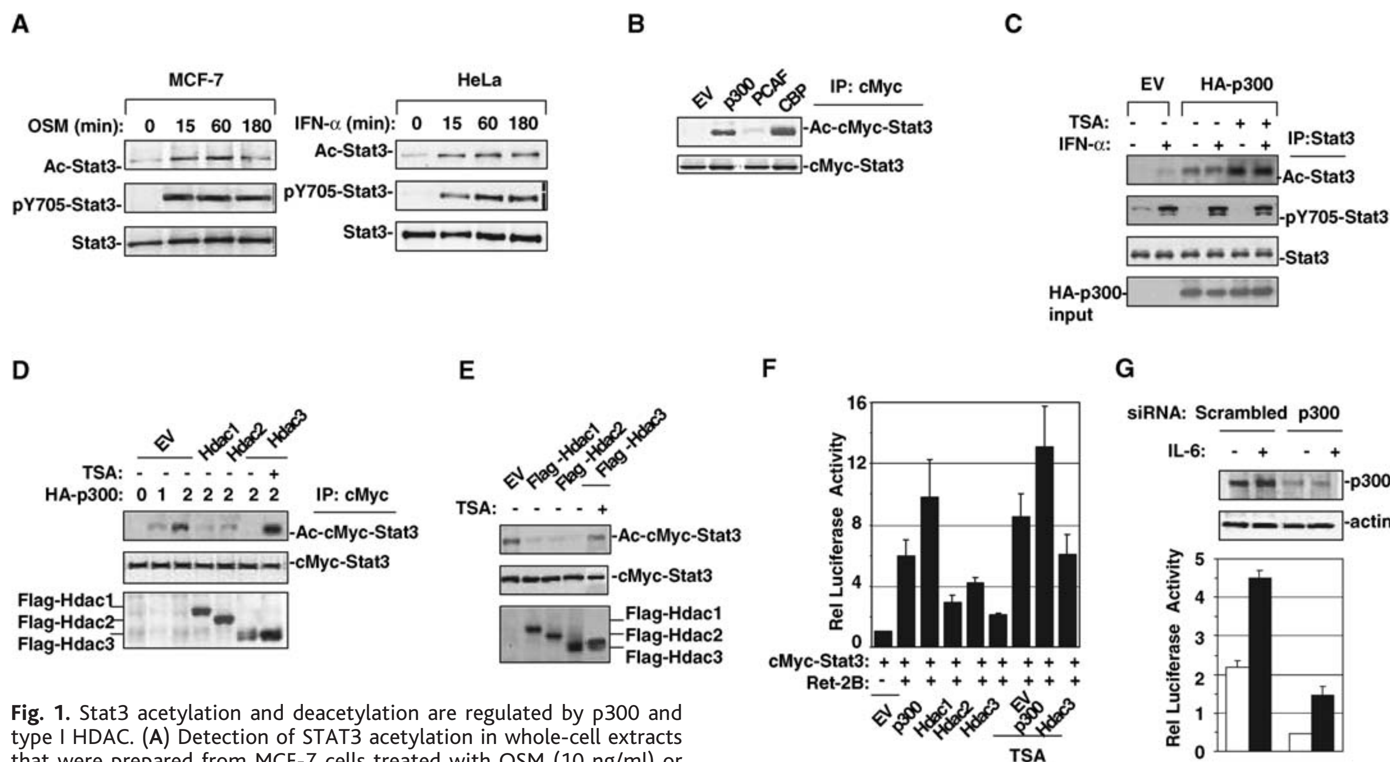


Fig. 1. Stat3 acetylation and deacetylation are regulated by p300 and type I HDAC. (**A**) Detection of STAT3 acetylation in whole-cell extracts that were prepared from MCF-7 cells treated with OSM (10 ng/ml) or from HeLa cells treated with IFN-α (500 U/ml). Lysates prepared from these cells were immunoprecipitated with anti-Stat3 and analyzed with antibodies to acetylated lysine (Cell Signaling Technology, Beverly, MA; catalog #9441), pY[705]-Stat3, and Stat3. (**B**) Stat3 acetylation by transfection of Stat3 with p300, PCAF, or CBP. Lysates from 293T cells transfected with Myc-tagged Stat3 and p300, PCAF, or CBP were immunoprecipitated (IP) with anti-cMyc and analyzed with antibodies to acetylated lysine or Stat3. (**C**) TSA augments Stat3 acetylation. 293T cells transfected with or without HA-p300 were incubated with 0.2 μM TSA (Sigma, St. Louis, MO) for 2 hours followed by IFN-α treatment for an additional 30 min. Anti-Stat3 immunoprecipitates from the whole-cell extracts were analyzed with antibodies to acetylated lysine, pY[705]-Stat3, Stat3, or HA. (**D**) Effect of type I HDAC on Stat3 acetylation in vivo. Whole-cell extracts were prepared from 293T cells that were transfected with HA-p300 at different doses (0, 1, 2 μg) or with 2 μg of HA-p300 DNA combined with 1 μg of vector encoding Hdac1, Hdac2, or Hdac3. Anti-cMyc immunoprecipitates were analyzed with antibodies to acetylated lysine or Stat3. In one condition, transfected 293T cells with p300 and Hdac3 were treated with 0.2 μM TSA for 2 hours. Expression levels of Hdac1, Hdac2, and Hdac3 were immunoblotted with anti-Flag (lower panel). (**E**) Stat3 deacetylation by type I HDAC in vitro. Acetyl-Stat3 proteins were immunoprecipitated with anti-cMyc from lysates of 293T cells transfected with p300 and cMyc-Stat3. Type I HDAC factors were immunoprecipitated with anti-Flag from 293T cells transfected with Flag-tagged Hdac1, Hdac2, or Hdac3. Stat3 deacetylation in vitro by type I HDAC was performed (*16*, *17*) and analyzed with antibodies to acetylated lysine, Stat3, or Flag. (**F**) Stat3 transcriptional activity was estimated in 293T cells transfected with p2xSIE-Luc, pRSV-β-gal, and RET-2B. In some samples, p300, Hdac1, Hdac2, and Hdac3 were included. Twenty-four hours after transfection, cells were treated with or without 0.2 μM TSA for 6 hours. The luciferase activity of each sample was normalized to β-galactosidase activity. Data are presented as means ± SD and represent results from three independent experiments. (**G**) p300 siRNA (AACCCCTCCTCTTCAGCACCA) or scramble siRNA was introduced into COS-1 cells for 24 hours, and SIE-luciferase reporter was transfected for an additional 24 hours followed by treatment with IL-6 for 6 hours. Whole extracts prepared from these cells were immunoblotted with antibodies to p300 or actin or analyzed for luciferase reporter activity. Data are presented as means ± SD and represent results from three independent experiments.

with the same sequence (fig. S3B). Additional evidence of Stat3 acetylation on Lys^{685} site was obtained by mass spectrometry analysis of acetylated Stat3 proteins purified from 293T cells transfected with Stat3 and p300 (*17*, *20*). Lys^{685} is highly conserved in Stat1, Stat3, Stat4, Stat5a, and Stat5b of different species. It resides in the highly hydrophilic region between the SH2 domain and TAD (fig. S3C). Secondary structural analysis predicts a small α helix immediately upstream of Lys^{685} residue and agrees with the crystal analysis of Stat1 (*3*).

To explore the role of Lys^{685} acetylation on Stat3 activity, we established cell lines by stably expressing either wild-type Stat3 or Stat3^{K685R} in PC3 cells, a human prostate cancer cell line lacking the *stat3* gene (*20*). Stat3^{K685R} was not acetylated, but both wild-type Stat3 and Stat3^{K685R} were tyrosine and serine phosphorylated and translocated into nuclei in response to OSM treatment (Fig. 4A and fig. S4). From analysis of the Stat1 and Stat3 crystal structures, we expected that the 35–amino acid segment between the SH2- and TAD-bearing Lys^{685} might be involved in Stat3 dimerization. Thus, we transfected cMyc-tagged and HA-tagged Stat3 into PC3 cells. Anti-cMyc immunoprecipitates from the whole-cell extracts prepared from these

Fig. 2. Interaction of Stat3 with p300 and Hdac3. (**A**) Whole extracts prepared from MCF-7 or HeLa cells were immunoprecipitated (IP) with antibodies to p300 (Santa Cruz Biotechnology) or control immunoglobulin G (IgG). Western blot analysis was performed with antibodies to Stat3 or p300. (**B**) Identification of the Stat3 domains interacting with p300. cMyc-Stat3 domain truncation mutants were transfected with HA-p300 in 293T cells. Anti-HA immunoprecipitates were analyzed by Western blot with antibodies to cMyc (upper panel) or HA. The expression of cMyc-Stat3 is shown in the bottom panel. (**C**) Stat3 associates with Hdac1, Hdac2, and Hdac3 in 293T cells. Anti-cMyc immunoprecipitates were subjected to Western blot analysis with antibodies to Flag or cMyc. (**D**) Identification of the Stat3 domains interacting with Hdac3. cMyc-Stat3 domain truncation mutants were transfected with Flag-Hdac3 in 293T cells. Anti-Flag-Hdac3 immunoprecipitates were analyzed by Western blot with anti-cMyc. (**E**) Identification of the Hdac3 domains interacting with Stat3. A series of Flag-Hdac3 with truncation mutations were constructed and transfected with cMyc-Stat3 into 293T cells. Anti-Flag immunoprecipitates were analyzed in Western blot with antibodies to cMyc or Flag.

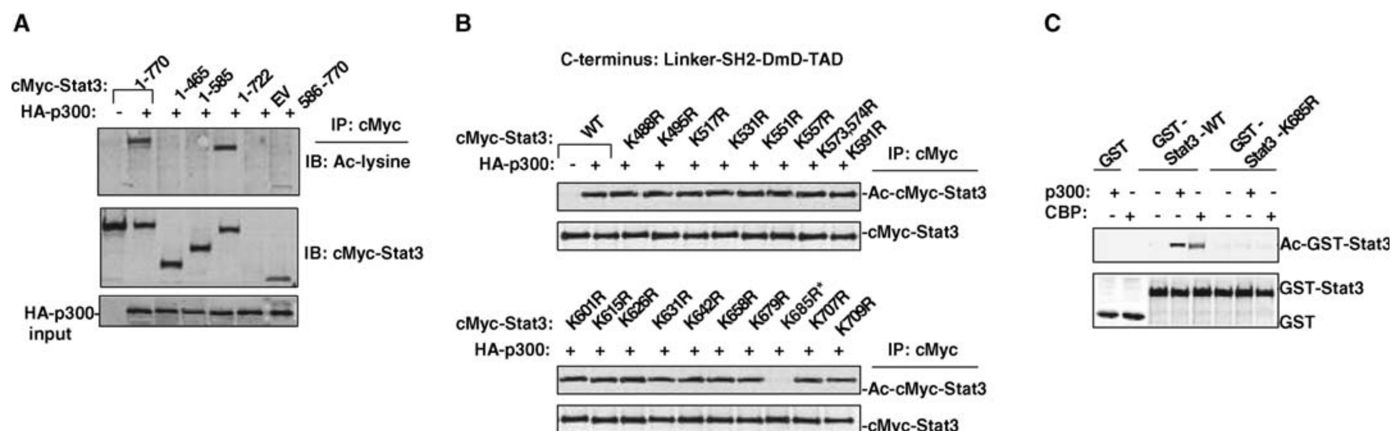


Fig. 3. Stat3 is acetylated on Lys^{685} site by p300. (**A**) Deletion analysis of Stat3 acetylation. A series of cMyc-tagged Stat3 with deletion mutations were cotransfected with HA-p300 in 293T cells. Anti-Stat3 immunoprecipitates were subjected to immunoblotting (IB) analysis with antibodies to acetyl-lysine or cMyc. HA-p300 levels are shown in the bottom panel. (**B**) p300-mediated acetylation of Stat3 with a K→R substitution. All Stat3 constructs with a K→R substitution were prepared by site-directed mutagenesis and transfected with p300 in 293T cells. cMyc-tagged Stat3 proteins were immunoprecipitated with anti-cMyc and analyzed with antibodies to acetyl lysine or cMyc. (**C**) K^{685}→R substitution abolishes STAT3 acetylation by p300 or CBP in vitro. Bacterial purified GST, wild-type GST-Stat3, or GST-Stat3-K^{685}R mutant proteins were incubated with purified p300 HAT protein domain or CBP protein in acetylation reaction buffer (*17*), followed by Western blot analysis with antibodies to acetylated lysine.

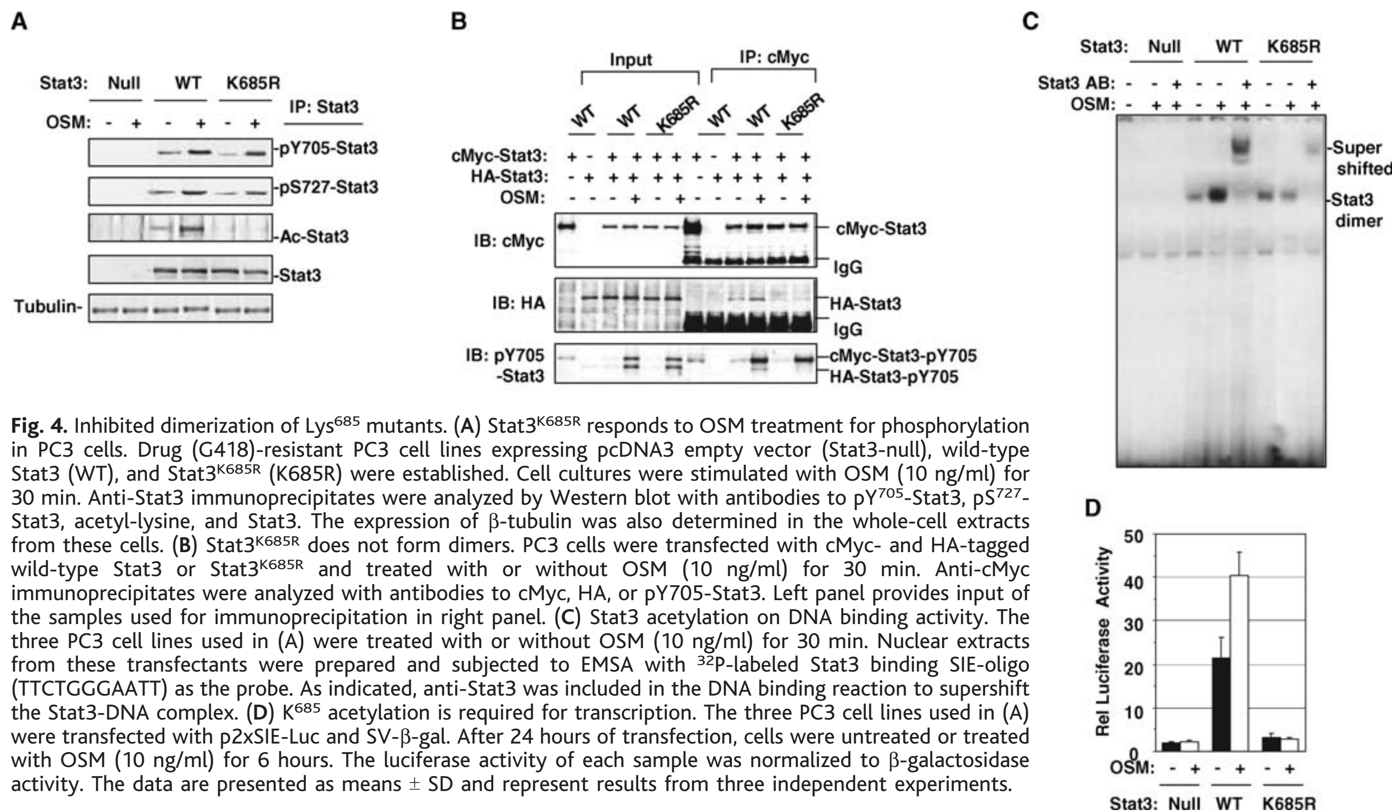
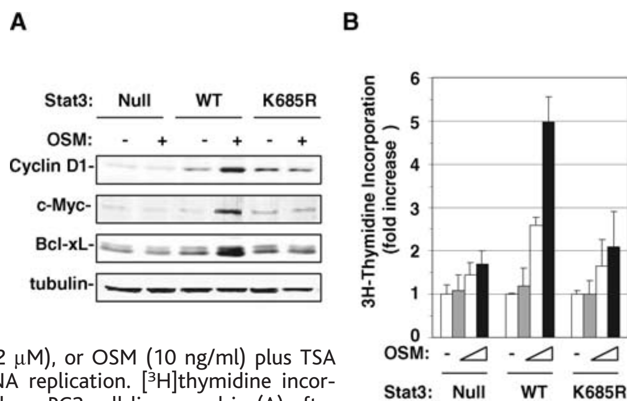


Fig. 4. Inhibited dimerization of Lys^{685} mutants. (**A**) Stat3^{K685R} responds to OSM treatment for phosphorylation in PC3 cells. Drug (G418)-resistant PC3 cell lines expressing pcDNA3 empty vector (Stat3-null), wild-type Stat3 (WT), and Stat3^{K685R} (K685R) were established. Cell cultures were stimulated with OSM (10 ng/ml) for 30 min. Anti-Stat3 immunoprecipitates were analyzed by Western blot with antibodies to pY^{705}-Stat3, pS^{727}-Stat3, acetyl-lysine, and Stat3. The expression of β-tubulin was also determined in the whole-cell extracts from these cells. (**B**) Stat3^{K685R} does not form dimers. PC3 cells were transfected with cMyc- and HA-tagged wild-type Stat3 or Stat3^{K685R} and treated with or without OSM (10 ng/ml) for 30 min. Anti-cMyc immunoprecipitates were analyzed with antibodies to cMyc, HA, or pY705-Stat3. Left panel provides input of the samples used for immunoprecipitation in right panel. (**C**) Stat3 acetylation on DNA binding activity. The three PC3 cell lines used in (A) were treated with or without OSM (10 ng/ml) for 30 min. Nuclear extracts from these transfectants were prepared and subjected to EMSA with ^{32}P-labeled Stat3 binding SIE-oligo (TTCTGGGAATT) as the probe. As indicated, anti-Stat3 was included in the DNA binding reaction to supershift the Stat3-DNA complex. (**D**) K^{685} acetylation is required for transcription. The three PC3 cell lines used in (A) were transfected with p2xSIE-Luc and SV-β-gal. After 24 hours of transfection, cells were untreated or treated with OSM (10 ng/ml) for 6 hours. The luciferase activity of each sample was normalized to β-galactosidase activity. The data are presented as means ± SD and represent results from three independent experiments.

PC3 transfectants were subjected to Western blot analysis with anti-HA or anti–tyrosine-phosphorylated Stat3 (Fig. 4B). In PC3 cells transfected with cMyc-tagged and HA-tagged wild-type Stat3, coimmunoprecipitation detected the association, which was stabilized by OSM treatment, between these two forms of Stat3 (Fig. 4B). However, when HA-Stat3^{K685R} and cMyc-Stat3^{K685R} were tested, the association between these two forms of Stat3 was undetectable, even though they were tyrosine phosphorylated upon OSM treatment (Fig. 4B). Thus, acetylation of Lys^{685} appears to be critical for Stat3 to form dimers. Because dimerized STAT proteins bind to DNA, we evaluated Stat3-DNA complex formation in these two cell lines using ^{32}P-labeled sis-inducible element (SIE) as the probe in electrophoretic mobility shift assay (EMSA). OSM treatment markedly induced formation of Stat3-DNA complex in PC3 cells expressing wild-type Stat3, whereas in PC3 cells expressing Stat3^{K685R}, we detected a basal level of complex formation, which was not increased in cells treated with OSM (Fig. 4C). Stat3-dependent luciferase activity assay revealed that introduction of wild-type Stat3 but not of Stat3^{K685R} restored the response of PC3 cells to OSM (Fig. 4D).

The effect of Stat3 acetylation on cell growth in vivo was further examined in PC3 cells transfected with empty vector, wild-type Stat3, or Stat3^{K685R}. Stat3 activation regulates genes involved in cell growth and cell survival, including *cyclin D1*, *bcl-X$_L$*, and c-*myc* (*21*, *22*). In parental PC3 cells or PC3 cells expressing empty vector, cyclin D1, cMyc, and Bcl-X$_L$ proteins were nearly undetectable in the presence or absence of OSM treatment (Fig. 5A). In PC3 cells expressing wild-type Stat3, OSM treatment stimulated the expression of these three proteins, as determined by Western blot analysis (Fig. 5A). In contrast, in PC3 cells expressing stable Stat3^{K685R}, OSM treatment did not alter the expression of these three proteins (Fig. 5A). TSA, the HDAC inhibitor, alone induced expression of cyclin D1, but only in PC3 cells expressing wild-type Stat3 (fig. S5). We also analyzed cell growth in these three types of PC3 cells. [^{3}H]thymidine incorporation assays revealed a dose-dependent stimulation of growth by OSM in wild-type Stat3-expressing PC3 cells, whereas parental cells or cells expressing Stat3^{K685R} showed little apparent effect of OSM on DNA replication (Fig. 5B). Consistent expression of wild-type Stat3 but not of Stat3-K^{685}R accelerated cell cycle progression in response to OSM treatment in PC3 cells after synchronization with aphidicolin (Fig. 5C). Inconsistent effects of Stat3 on cell growth in different cell types have been reported (*21–23*), but our results demonstrate that expression of Stat3 is required for transcriptional activation of genes involved in cell cycle progression and for stimulating cell growth after exposure to OSM in PC3 cells.

GKX$_2$P (where G is glycine, P is proline, and X represents any amino acid), or GK, is a preferred sequence in histone or other protein substrates for acetylation by HAT (*19*, *24*). In STAT, the acetylated lysine is relatively conserved as a G(S)KX$_{3-5}$P sequence. Recently, genes carrying STAT-type linker-SH2 domains within their C-terminal regions have been discovered in *Arabidopsis*, indicating that the linker-SH2 domain of STATs was fully developed before the divergence of plant and animal kingdoms (*25*). In animals, the linker-SH2 domain of STAT extends into DmD, which harbors the GKX$_{3-5}$P motif and the phosphotyrosine motif GYXK followed by TAD, which harbors the phosphoserine motif PMSP. Stat2 and Stat6 represent the most divergent STAT members in evolution (*26*), and both lack GKX$_{3-5}$P and PMSP motifs in the C-terminal region. It is possible that another lysine residue within this domain is acetylated in these two STATs. Although in animals STAT evolved to have DmD-TAD, both GKX$_{3-5}$P and PMSP motifs might have evolved after the appearance of the GYXK motif. STAT acetylation may well be analogous to STAT tyrosine phosphorylation. However, neither Tyr^{705} phosphorylation alone nor Lys^{685} acetylation alone seems to be sufficient for Stat3 activation. Acetylation of Lys^{685} may change the local charge of DmD, form a platform for the interaction, and strengthen the dimer formation (fig. S6). Crystal analysis revealed that STAT (Stat1 and Stat3), nuclear factor NF-κB (Rel A), and p53 bind DNA in a similar topology (*2*, *3*). In p53, C-terminal acetylation regulates p53 DNA binding activity, presumably via a



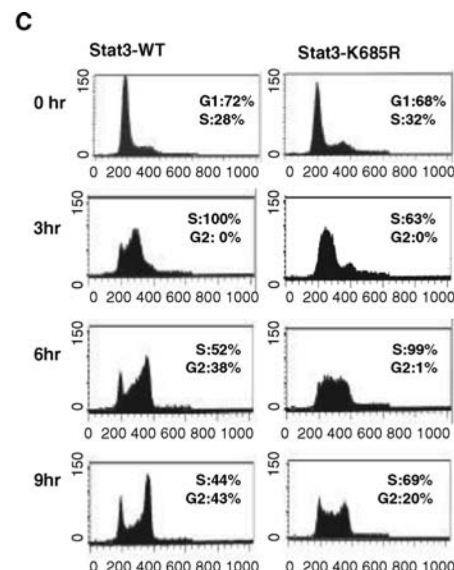


Fig. 5. Lys^{685} acetylation in Stat3 stimulates cell proliferation. (**A**) Lys^{685} acetylation is critical for cell cycle–related gene expression. Western blot analysis was performed with antibodies to cyclin D1 (Santa Cruz Biotechnology), cMyc (Santa Cruz Biotechnology), Bcl-$X_{S/L}$ (Santa Cruz Biotechnology), and β-tubulin in PC3 cells expressing empty vector (null), wild-type Stat3 (WT), or Stat3^{K685R} (K685R) treated with OSM (10 ng/ml), TSA (0.2 μM), or OSM (10 ng/ml) plus TSA (0.2 μM) for 12 hours. (**B**) DNA replication. [^{3}H]thymidine incorporation was analyzed in the three PC3 cell lines used in (A) after OSM (5, 10, and 20 ng/ml) treatment for a period of 24 hours (*17*). Data are presented as means ± SD and represent results from three independent experiments. (**C**) Accelerated S-phase entry by expression of wild-type Stat3 but not Stat3^{K685R}. PC3 cells (2×10^5) expressing either wild-type Stat3 or Stat3-K^{685}R mutant were synchronized with aphidicolin (2 μg/ml) for a period of 12 hours, released from aphidicolin, and then treated with OSM for the times as indicated. Flow cytometric analysis was subsequently performed with cells treated in this manner (*17*).

conformational change of p53 (*18*). In Rel A, acetylation of lysine sites in the dimerization domain augments Rel A's DNA binding activity (*27*). Our results raise the possibility that acetylation of NF-κB and p53 promotes transcriptional activity by a mechanism similar to the acetylation of Stat3. In HeLa cells, all three type I HDACs are associated with Stat3, either in the cytoplasm or nucleus. As such, unlike NF-κB, which responds only to Hdac-3 for deacetylation (*28*), type I HDACs function as Stat3-deacetylase factors that, when associated with Stat3, either terminate Stat3's stimulatory effect on transcription or maintain Stat3 in a deacetylated form. Given the role of CBP/p300 as a coactivator for different STAT members, it is possible that all STAT family members are tightly regulated by the acetylation and deacetylation cycle.

References and Notes

1. N. Stahl *et al.*, *Science* **267**, 1349 (1995).
2. S. Becker, B. Groner, C. W. Muller, *Nature* **394**, 145 (1998).
3. X. Chen *et al.*, *Cell* **93**, 827 (1998).
4. K. Shuai *et al.*, *Cell* **76**, 821 (1994).
5. J. Braunstein, S. Brutsaert, R. Olson, C. Schindler, *J. Biol. Chem.* **278**, 34133 (2003).
6. A. Kumar, M. Commane, T. W. Flickinger, C. M. Horvath, G. R. Stark, *Science* **278**, 1630 (1997).
7. N. V. Zhukovskaya *et al.*, *Development* **131**, 447 (2004).
8. A. K. Kretzschmar, M. C. Dinger, C. Henze, K. Brocke-Heidrich, F. Horn, *Biochem. J.* **377**, 289 (2004).
9. S. Bhattacharya *et al.*, *Nature* **383**, 344 (1996).
10. J. J. Zhang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 15092 (1996).
11. M. Paulson *et al.*, *J. Biol. Chem.* **274**, 25343 (1999).
12. E. Korzus *et al.*, *Science* **279**, 703 (1998).
13. R. H. Goodman, S. Smolik, *Genes Dev.* **14**, 1553 (2000).
14. P. Z. Yuan, Y. E. Chin, unpublished data.
15. I. Nusinzon, C. M. Horvath, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 14742 (2003).
16. W. M. Yang, S. C. Tsai, Y. D. Wen, G. Fejer, E. Seto, *J. Biol. Chem.* **277**, 9447 (2002).
17. Materials and methods are available as supporting material on *Science* Online.
18. W. Gu, R. G. Roeder, *Cell* **90**, 595 (1997).
19. M. A. Martinez-Balbas, U. M. Bauer, S. J. Nielsen, A. Brehm, T. Kouzarides, *EMBO J.* **19**, 662 (2000).
20. J. Clark *et al.*, *Oncogene* **22**, 1247 (2003).
21. J. F. Bromberg *et al.*, *Cell* **98**, 295 (1999).
22. T. Matsui, T. Kinoshita, T. Hirano, T. Yokota, A. Miyajima, *J. Biol. Chem.* **277**, 36167 (2002).
23. C. K. Lee *et al.*, *Immunity* **17**, 63 (2002).
24. J. R. Rojas *et al.*, *Nature* **401**, 93 (1999).
25. Q. Gao *et al.*, *Mol. Cell. Proteomics* **3**, 704 (2004).
26. A. H. Brivanlou, J. E. Darnell Jr., *Science* **295**, 813 (2002).
27. L. F. Chen, Y. Mu, W. C. Greene, *EMBO J.* **21**, 6539 (2002).
28. L. F. Chen, W. Fischle, E. Verdin, W. C. Greene, *Science* **293**, 1653 (2001).

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Methods
Figs. S1 to S6

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