restore LexA-VP16 destruction in *Met30*-null cells (Fig. 3A), the fusion did rescue transcriptional activation, restoring wild-type levels of VP16 TAD activity (Fig. 3B, compare VP16 and Ub-VP16 in the *Met30*-null cells). Moreover, both Ub and the VP16 TAD are required for transcriptional activation in the *Met30*-null cells because Ub alone fused to LexA (Ub-Δ) did not activate transcription (*14*). The observation that Met30's role in transcription can be complemented by fusion of LexA-VP16 to Ub argues that Met30 coactivates the VP16 TAD by signaling LexA-VP16 ubiquitylation. Moreover, the metabolic stability of the Ub-VP16 protein (Fig. 3A) demonstrates that it is Met30-mediated ubiquitylation, not destruction, that is required for transcriptional activation.

The requirement of ubiquitylation for VP16 activator function reveals that the degron function of the VP16 TAD is intimately tied to its ability to activate transcription. The link between these processes provides a simple explanation for the frequent and intimate overlap of TADs and degrons (*3*–*5*). This requirement for ubiquitylation, which has not been observed in vitro, reveals a function for Ub distinct from its role in proteolysis (*2*). Recent evidence has demonstrated that the 19S subunit of the proteasome plays an essential role in transcriptional elongation (*15*). Given the role of the 19S complex as a Ub binding module (*16*), it is possible that activator ubiquitylation serves to recruit the 19S complex to promoters, where the chaperone functions of this complex promote transcription elongation.

Although our data demonstrate that proteolysis is not required for transcriptional activation, it is important to note that Met30 does direct LexA-VP16 destruction. This suggests that activator destruction by the proteasome is a natural consequence of ubiquitylation. Because of the dual role of Ub in transcriptional activation and activator destruction, therefore, we propose that Ub "licenses" transcription factors by linking their activity to their destruction. We imagine that non-ubiquitylated activators are stable and inactive. Interactions of an activator with a Ub-ligase result in activator ubiquitylation, which simultaneously activates the transcription factor and primes it for destruction by the proteasome. Given the large number of transcription factors that are targeted for Ub-mediated proteolysis, it is possible that many transcription factors are regulated through this mechanism.

References and Notes

1. D. Thomas, M. Tyers, *Curr. Biol.* **10**, R341 (2000).
2. A. Varshavsky, *Trends Biochem. Sci.* **22**, 383 (1997).
3. S. E. Salghetti, S. Y. Kim, W. P. Tansey, *EMBO J.* **18**, 717 (1999).
4. E. Molinari, M. Gilman, S. Natesan, *EMBO J.* **18**, 6439 (1999).
5. S. E. Salghetti, M. Muratani, H. Wijnen, B. Futcher, W. P. Tansey, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 3118 (2000).
6. S. J. Triezenberg, R. C. Kingsbury, S. L. McKnight, *Genes Dev.* **2**, 718 (1988).
7. R. Brent, M. Ptashne, *Cell* **43**, 729 (1985).
8. D. Skowyra, K. L. Craig, M. Tyers, S. J. Elledge, J. W. Harper, *Cell* **91**, 209 (1997).
9. Because the VP16 TAD contains no lysine residues, LexA-VP16 ubiquitylation probably occurs within the LexA protein.
10. A. Barberis, *et al*., *Cell* **81**, 359 (1995).
11. *Met30* (–) cells expressing LexA–VP16 grow at an identical rate to *Met30* (+) cells expressing this protein. The indistinguishable growth rates of these cells support the argument that LexA-VP16 does not globally suppress (i.e., squelch) transcription in the *Met30*-null background.
12. R. Li *et al*., *Mol. Cell. Biol.* **18**, 1296 (1998).
13. Y. Marahrens, B. Stillman, *Science* **255**, 817 (1992).
14. The finding that the VP16 TAD is still required for transcriptional activation in this setting demonstrates that VP16 TAD function is not globally suppressed in the absence of Met30. Together with the results of the plasmid stability analysis (Fig. 2C), this finding demonstrates that the VP16 TAD retains at least part of its activity in the *Met30*-null cells.
15. A. Ferdous, F. Gonzalez, L. Sun, T. Kodadek, S. A. Johnston, *Mol. Cell* **7**, 981 (2001).
16. D. Voges, P. Zwickl, W. Baumeister, *Annu. Rev. Biochem.* **68**, 1015 (1999).
17. E. E. Patton *et al*., *EMBO J.* **19**, 1613 (2000).
18. A. Rouillon, R. Barbey, E. E. Patton, M. Tyers, D. Thomas, *EMBO J.* **19**, 282 (2000).
19. E. S. Johnson, P. C. Ma, I. M. Ota, A. Varshavsky, *J. Biol. Chem.* **270**, 17442 (1995).
20. Sequences encoding the VP16, Myc, and Cln3 degrons were inserted into pRJR238 (*10*), modified to include an in-frame hemagglutinin (HA)-epitope tag (pRJR238HA). The resulting LexA-fusion proteins were expressed in CC849-1B (*MAT* **a**, *his3*, *leu2*, *ura3*, *trp1*, *met4::TRP1*) and CC807-7D (*MAT* **a**, *ade2*, *his3*, *leu2*, *ura3*, *trp1*, *met4::TRP1*, *met30::LEU2*) yeast (*17*), each carrying an integrated LexA–β-galactosidase reporter (*10*). Pulse-chase analysis was performed as described (*3*).
21. LexA fusion proteins were expressed in CC849-1B, along with either glutathione *S*-transferase (GST) or GST-Met30 (*18*) under the control of the GAL1 promoter. Log-phase cultures were induced with galactose, proteins were harvested, and GST proteins were collected on glutathione agarose (*18*).
22. J. Spence *et al*., *Cell* **102**, 67 (2000).
23. LexA-VP16 appears to be modified in *Met30* (+) cells. This modification is not due to ubiquitylation because that species of LexA-VP16 does not react with antibodies to ubiquitin.
24. A. Hecht, M. Grunstein, *Methods Enzymol.* **304**, 399 (1999).
25. CC894-1B and CC807-7D, which carry the indicated pRJR238HA plasmids, were transformed with pARS1-LEXA, which carries a *URA3* marker and a modified LexA-ARS1 DNA replication origin. Plasmid stability was measured as described (*13*).
26. Ub coding sequences were polymerase chain reaction (PCR)–amplified from yeast genomic DNA with two modifications: (i) the carboxy-terminal glycine residue was changed to alanine to prevent removal of the Ub moiety by isopeptidases (*19*), and (ii) the T7-epitope tag sequence was added at the carboxy terminus of Ub.

27. For reagents, we thank D. Finley, W. Herr, R. Li, M. Ptashne, and D. Thomas. We thank S. Grewal, R. Li, A. Matapurkar, and V. Valmeekam for technical help. A.A.C. is a George A. and Marjorie H. Anderson Fellow of the Watson School of Biological Sciences and a Howard Hughes Medical Institute Predoctoral Fellow. W.P.T. is a Kimmel Scholar. Supported by the Cold Spring Harbor Laboratory Cancer Center Support Grant CA45508 and by USPHS grant CA-13106 from the National Cancer Institute.

30 April 2001; accepted 2 July 2001
Published online 19 July 2001;
10.1126/science.1062079
Include this information when citing this paper.

Duration of Nuclear NF-κB Action Regulated by Reversible Acetylation

Lin-feng Chen,[1] Wolfgang Fischle,[1] Eric Verdin,[1,2] Warner C. Greene[1,2,3]*

The nuclear expression and action of the nuclear factor kappa B (NF-κB) transcription factor requires signal-coupled phosphorylation and degradation of the IκB inhibitors, which normally bind and sequester this pleiotropically active factor in the cytoplasm. The subsequent molecular events that regulate the termination of nuclear NF-κB action remain poorly defined, although the activation of de novo IκBα gene expression by NF-κB likely plays a key role. Our studies now demonstrate that the RelA subunit of NF-κB is subject to inducible acetylation and that acetylated forms of RelA interact weakly, if at all, with IκBα. Acetylated RelA is subsequently deacetylated through a specific interaction with histone deacetylase 3 (HDAC3). This deacetylation reaction promotes effective binding to IκBα and leads in turn to IκBα-dependent nuclear export of the complex through a chromosomal region maintenance-1 (CRM-1)–dependent pathway. Deacetylation of RelA by HDAC3 thus acts as an intranuclear molecular switch that both controls the duration of the NF-κB transcriptional response and contributes to the replenishment of the depleted cytoplasmic pool of latent NF-κB–IκBα complexes.

NF-κB corresponds to an inducible transcription factor complex that plays a pivotal role in regulating the inflammatory, immune, and anti-apoptotic responses in mammals (*1*, *2*). The prototypical NF-κB complex, which corresponds to a heterodimer of p50 and RelA subunits, is sequestered in the cytoplasm by its assembly with a family of inhibitory proteins termed the IκBs (*1*). Stimulus-induced phosphorylation of two NH_2-terminal serines in the IκBs, mediated by a macromolecular IκB kinase complex (IKK) (*3*), triggers the

rapid ubiquitination and subsequent degradation of this inhibitor by the 26S proteasome complex. The liberated NF-κB heterodimer rapidly translocates into the nucleus, where it engages cognate κB enhancer elements and alters gene expression. The NF-κB complex also recruits the p300/CBP and P/CAF coactivators, which participate in the activation of target gene transcription (*4–6*). Phosphorylation of an NH_2-terminal site in RelA by protein kinase A facilitates NF-κB assembly with CBP/p300 (*7*). Both CBP and p300 contain histone acetyltransferase activity that has been implicated in the regulation of gene expression. These effects involve acetylation of core histones leading to changes in chromatin structure (*8–13*) as well as direct acetylation of select host transcription factors like

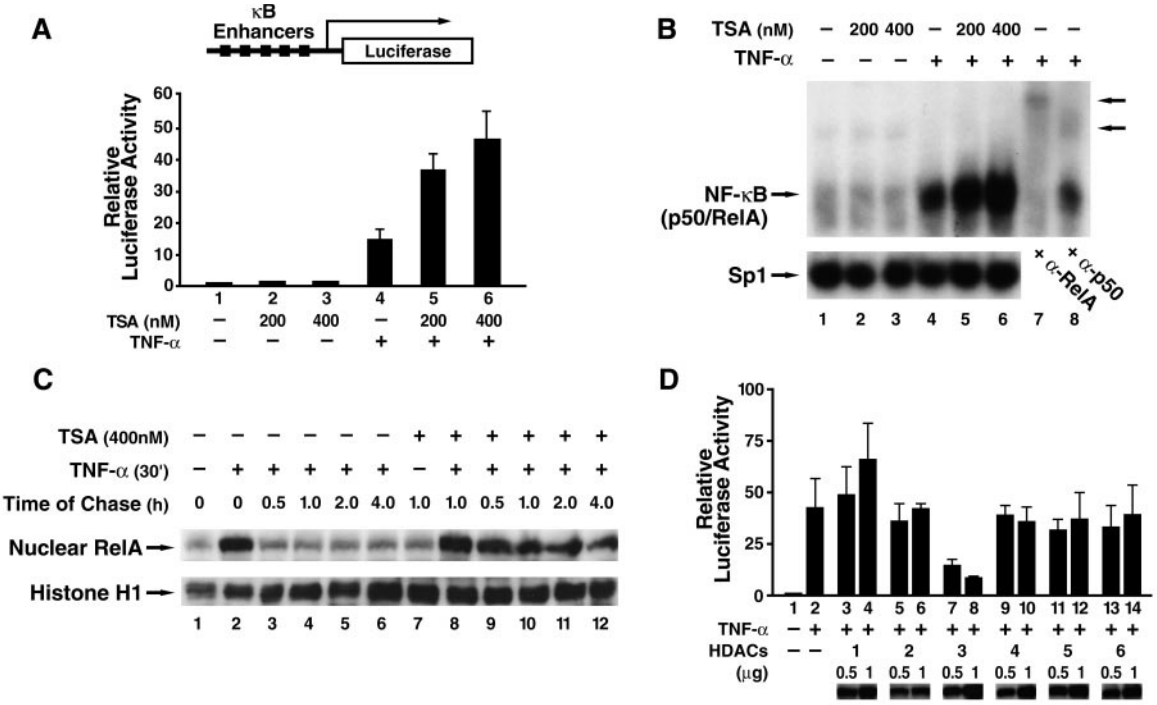

Fig. 1. (**A**) TSA enhances TNF-α–mediated activation of κB-luciferase gene expression. 293T cells were transfected with κB-luciferase reporter plasmid DNA (0.1 μg) and luciferase activity was measured (*19*) after treatment with TSA and TNF-α (10 ng/ml). Results represent cumulated data from three independent transfections. (**B**) TSA enhances TNF-α induction of nuclear NF-κB DNA-binding activity. NF-κB DNA-binding activity was assessed in electrophoretic mobility shift assays (EMSA) with a ^{32}P-radiolabeled consensus κB enhancer oligonucleotide (5′-AGTTGAGGGGACTTTCCCAGGC-3′) (upper panel). Supershifting this complex with anti-RelA (SC-109, Santa Cruz Biotechnology, Santa Cruz, CA) and anti-p50 (SC-1190, Santa Cruz) is shown in lanes 7 and 8. Comparability of the various nuclear extracts was assessed by EMSA with a ^{32}P-radiolabeled Sp1 probe (Promega) (lower panel). (**C**) RelA expression is sustained in the nuclei of TNF-α–stimulated cells in the presence of TSA. HeLa cells were incubated in the presence (lanes 7 to 12) or absence (lanes 1 to 6) of TSA (400 nM) for 1 hour before stimulation with TNF-α (20 ng/ml) for 30 min. The cells were chased for the indicated time periods with medium alone (lanes 1 to 6) or medium containing TSA (400 nM) (lanes 7 to 12). Nuclear extracts from each culture were immunoblotted with anti-RelA and visualized with an enhanced chemiluminescence reagent (Amersham). (**D**) HDAC3, but not HDAC1, 2, 4, 5, or 6, inhibits TNF-α activation of κB-luciferase activity. Expression levels of each HDAC determined by anti-Flag immunoblotting of the cell lysates are shown in the insets.

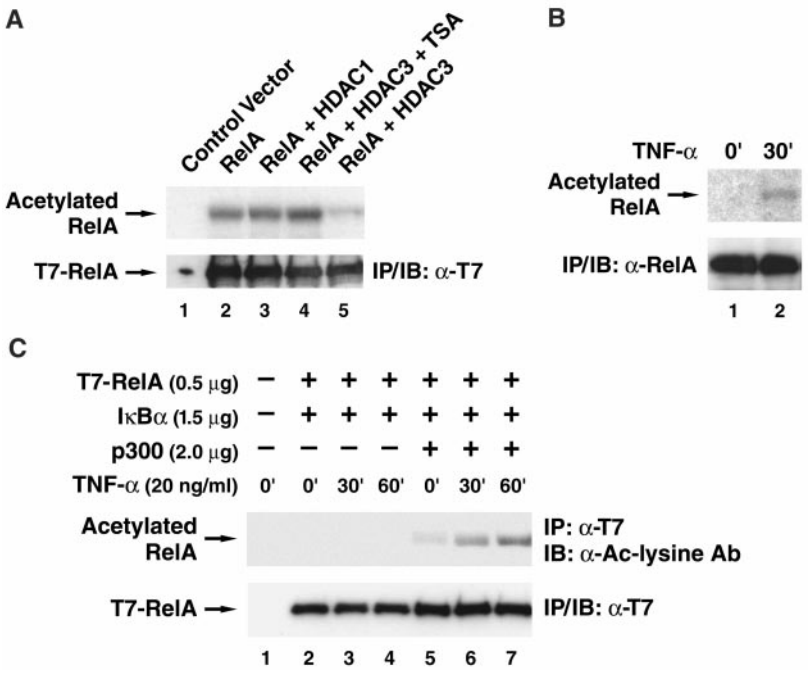

Fig. 2. (**A**) RelA is acetylated in vivo. COS-7 cells were cotransfected with expression vectors encoding T7-RelA and HDAC1 or HDAC3. Acetylation levels of RelA determined by [^{3}H]sodium acetate labeling (*19*) are shown in the upper panel. The total amount of T7-RelA in the individual samples is shown in the lower panel. (**B**) Endogenous RelA is inducibly acetylated after TNF-α stimulation. Acetylation of endogenous RelA was assessed by [^{3}H]sodium acetate labeling (*19*) and is shown in the upper panel. The level of RelA present in each of the two immunoprecipitates is shown in the lower panel. (**C**) RelA is inducibly acetylated by p300 in vivo. 293T cells were cotransfected with T7-RelA and IκBα in the presence or absence of expression plasmids encoding p300. Cell cultures were stimulated with TNF-α for the indicated time periods, and acetylation levels of RelA were analyzed by immunoblotting with antibody to acetylated lysine (Cell Signaling, Beverly, MA). The level of T7-RelA present in each of the immunoprecipitates is also shown in the lower panel.

p53, GATA-1, and E2F, which alters their biological function (*9*, *10*).

Although much progress has been made in understanding the biochemical events that underlie NF-κB induction, much less is known about how activated nuclear forms of NF-κB are negatively regulated, ensuring a transient transcriptional response. Prior studies have demonstrated that the IκBα gene is induced by NF-κB (*14*–*16*) and that the de novo expression of IκBα proteins, which display nucleocytoplasmic shuttling properties, participates in a negative feedback system limiting the NF-κB transcriptional response (*17*, *18*). We now demonstrate that nuclear RelA is subject to reversible acetylation and that this posttranslational modification plays a pivotal role in NF-κB regulation by governing IκBα binding to RelA and the nuclear export of the NF-κB complex.

[1]Gladstone Institute of Virology and Immunology, [2]Department of Medicine, [3]Department of Microbiology and Immunology, University of California, San Francisco, CA 94141, USA.

*To whom correspondence should be addressed. E-mail: wgreene@gladstone.ucsf.edu

We first observed that trichostatin A (TSA), a specific inhibitor of the multiple histone deacetylases (HDACs) (*11*), enhanced tumor necrosis factor (TNF)-α–induced, but not basal, expression of a κB-luciferase reporter gene (Fig. 1A). In addition, TSA enhanced nuclear NF-κB DNA binding after TNF-α stimulation but did not itself stimulate NF-κB binding (Fig. 1B). TSA also did not alter the binding of the constitutively expressed Sp1 transcription factor in either the presence or absence of TNF-α (Fig. 1B, lower panel). Immunoblotting of the nuclear extracts with antibodies to RelA (anti-RelA) revealed sustained nuclear RelA expression in the presence of TSA (Fig. 1C). Together, these results indicate that TSA enhances TNF-α–induced NF-κB DNA binding, likely by prolonging intranuclear expression of RelA.

TSA broadly inhibits the action of the HDACs. HDACs function not only to deacetylate core histones leading to repressive changes in chromatin structure but also to deacetylate various host transcription factors, altering their transcriptional activity (*10*–*13*). We assessed potential inhibitory effects of various HDACs on NF-κB action by cotransfecting 293T cells with expression vectors encoding HDAC1, 2, 3, 4, 5, or 6 and a κB-luciferase reporter followed by stimulation with TNF-α for 5 hours (Fig. 1D). Although each of the HDACs was comparably expressed, only HDAC3 inhibited TNF-α–induced κB-luciferase activity (lanes 7 and 8). This inhibitory effect of HDAC3 was abrogated in the presence of TSA, indicating that the deacetylase function of HDAC3 was required for these biological effects (Web fig. 1) (*19*). Consistent with this action of HDAC3, we found that the RelA subunit of NF-κB is acetylated in vivo (Fig. 2A, lane 2, upper panel). RelA acetylation was abolished in the presence of HDAC3 (lane 5) but not HDAC1 (lane 3). The addition of TSA to the HDAC3-expressing cultures was associated with the detection of acetylated RelA (lane 4). When HeLa cells were incubated in [^{3}H]sodium acetate and stimulated with TNF-α or medium for 30 min, signal-induced acetylation of endogenous RelA was similarly detected (Fig. 2B). Together, these studies reveal that NF-κB is subject to reversible acetylation and that HDAC3 plays a central role in its deacetylation.

The potential participation of the p300,

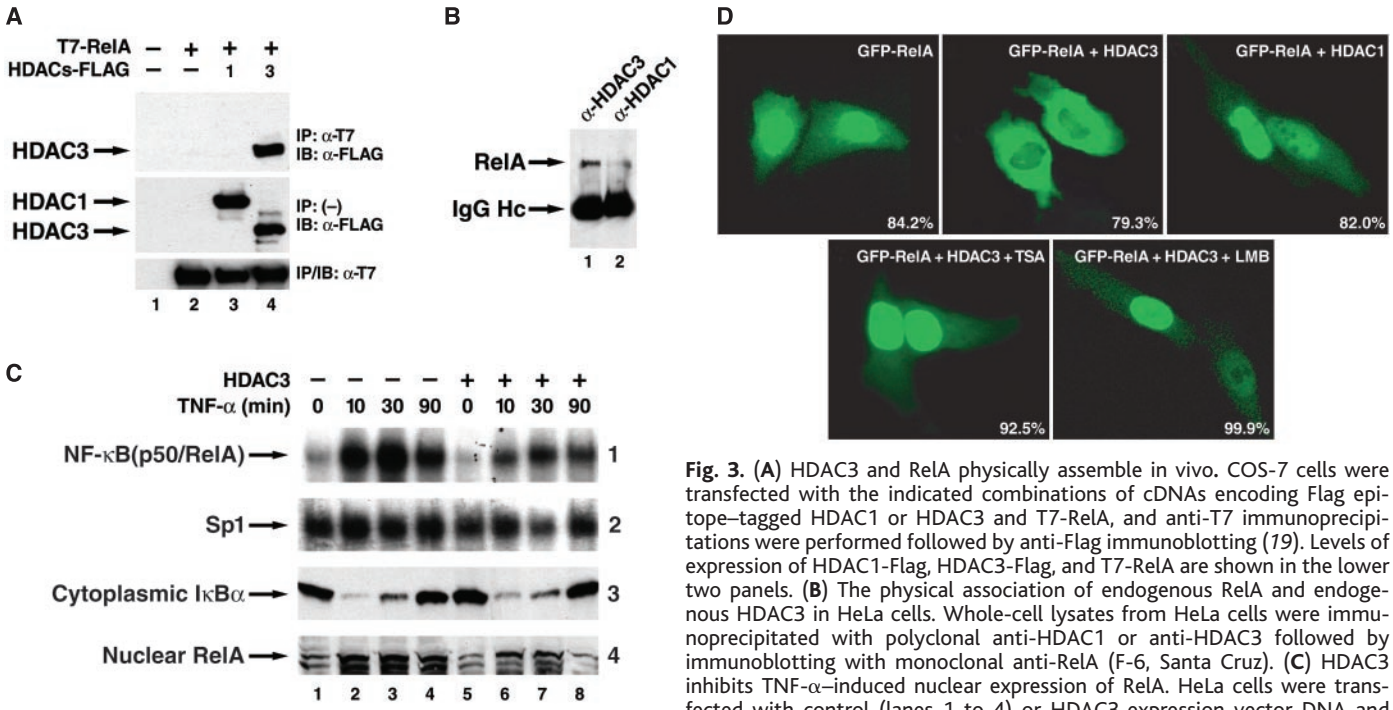

Fig. 3. (**A**) HDAC3 and RelA physically assemble in vivo. COS-7 cells were transfected with the indicated combinations of cDNAs encoding Flag epitope–tagged HDAC1 or HDAC3 and T7-RelA, and anti-T7 immunoprecipitations were performed followed by anti-Flag immunoblotting (*19*). Levels of expression of HDAC1-Flag, HDAC3-Flag, and T7-RelA are shown in the lower two panels. (**B**) The physical association of endogenous RelA and endogenous HDAC3 in HeLa cells. Whole-cell lysates from HeLa cells were immunoprecipitated with polyclonal anti-HDAC1 or anti-HDAC3 followed by immunoblotting with monoclonal anti-RelA (F-6, Santa Cruz). (**C**) HDAC3 inhibits TNF-α–induced nuclear expression of RelA. HeLa cells were transfected with control (lanes 1 to 4) or HDAC3-expression vector DNA and cultured for 24 hours, followed by stimulation with TNF-α (10 ng/ml) for 0, 10, 30, or 90 min. Nuclear extracts were prepared and EMSAs performed as described in Fig. 1B (panels 1 and 2). TNF-α–induced degradation of IκBα was analyzed in the cytoplasmic extracts of these cultures by immunoblotting with anti-IκBα (C-21, Santa Cruz) (panel 3). Levels of nuclear RelA were assessed by immunoblotting nuclear extracts with specific anti-RelA (panel 4). The faster migrating bands reactive with the anti-RelA likely correspond to RelA degradation products and show similar changes in levels of nuclear expression. (**D**) HDAC3 stimulates nuclear export of RelA via a leptomycin B–sensitive pathway. GFP-RelA principally localized in the nucleus when expression plasmid DNA was transfected into HeLa cells. Coexpression of HDAC3 and GFP-RelA resulted in a cytoplasmic expression pattern of GFP-RelA; HDAC3 and GFP-RelA plasmid DNA was transfected at a 6:1 ratio to ensure that all GFP-RelA–expressing cells also contained HDAC3. Coexpression of HDAC1 and GFP-RelA did not alter the nuclear pattern of GFP-RelA epifluorescence. Treatment of the GFP-RelA and HDAC3–expressing cell cultures with TSA (800 nM, 5 hours) resulted in a nuclear pattern of expression of GFP-RelA. Treatment of GFP-RelA and HDAC3–expressing cultures with leptomycin B (LMB; 20 nM, 2 hours) produced a nuclear pattern of GFP-RelA epifluorescence. Average percentages of cells displaying the depicted phenotype are shown in the lower right corner of each panel. Results are derived from inspection of at least 200 transfected cells present in multiple microscopic fields from two independent experiments.

CBP, and P/CAF acetyltransferases in the acetylation of RelA was next investigated. When 293T cells were cotransfected with RelA, IκBα, and p300, and stimulated with TNF-α, inducible acetylation of RelA was detected (Fig. 2C). Coexpression of either p300 or CBP, but not P/CAF, with RelA produced a dose-dependent acetylation of T7-RelA [Web fig. 2 (*19*)]. Together, these findings demonstrate that endogenous RelA is acetylated in a signal-coupled manner likely mediated by p300 and CBP.

Coimmunoprecipitation of T7-RelA and HDAC3 in COS-7 cells suggests a physical interaction between these two proteins in vivo (Fig. 3A). In contrast, no interaction between RelA and HDAC1 was detected despite comparable levels of both HDACs being expressed (Fig. 3A). Moreover, anti-HDAC3 effectively coimmunoprecipitated endogenous RelA from HeLa cells, whereas only trace amounts of RelA immunoprecipitated with anti-HDAC1 (Fig. 3B). By using coimmunoprecipitation assays and the mammalian two-hybrid system (*20*), we found that NH_2-terminal regions of both HDAC3 and RelA are required for the assembly of these two proteins in vivo [Web fig. 3) (*19*)].

Studies were next performed to define the molecular basis for HDAC3-mediated inhibition of TNF-α activation of NF-κB. Expression of HDAC3 in HeLa cells (~50% transfection efficiency) diminished both NF-κB DNA-binding activity (Fig. 3C, panel 1) and levels of nuclear RelA (panel 4). In contrast, HDAC3 expression did not alter TNF-α–induced degradation of IκBα occurring in the cytoplasm (panel 3), nor did it markedly change the levels of Sp1 DNA-binding activity (panel 2). Together with the results presented in Fig. 1C, these findings raise the possibility that HDAC3 may regulate the intracellular trafficking of RelA. A precedent for acetylation influencing the intracellular trafficking of a transcription factor is provided by recent studies on hepatocyte nuclear factor-4 and class II transactivator (CIITA) (*21*, *22*). To monitor potential effects of HDAC3 on the intracellular trafficking of RelA, we expressed green fluorescent protein–RelA fusion proteins (GFP-RelA) in HeLa cells in the presence and absence of HDAC3 (Fig. 3D). When expressed alone or with HDAC1, GFP-RelA localized to the nucleus, whereas the coexpression of HDAC3 resulted in a predominantly cytoplasmic pattern of GFP-RelA expression. This cytoplasmic relocalization of GFP-RelA induced by HDAC3 did not occur in the presence of TSA. These effects of HDAC3 appeared to result from nuclear export of GFP-RelA because the addition of leptomycin B, a known inhibitor of CRM-1/exportin-1–dependent nuclear export (*23*), preserved the nuclear pattern of GFP-RelA epifluorescence. These findings suggest that HDAC3-mediated deacetylation of RelA promotes its export from the nucleus to the cytoplasm.

Among the target genes activated by NF-κB is the IκBα gene (*14*–*16*). The resultant de novo synthesis of IκBα serves to replenish the intracellular stores of this inhibitor depleted during the course of NF-κB activation. IκBα also displays nucleocytoplasmic shuttling properties and likely retrieves nuclear NF-κB complexes, thereby contributing to the termination of the NF-κB transcriptional response (*17*, *18*). We next examined whether the acetylation status of RelA regulates its assembly with IκBα. The acetylation of RelA induced by the coexpression of increasing amounts of p300 was associated with markedly diminished binding of RelA to GST-IκBα matrices (Fig. 4A, lanes 3 and 4). However, the coexpression of increasing amounts of HDAC3 in the presence of p300 restored RelA binding to GST-IκBα (Fig. 4A, lanes 5 and 6). Immunoblotting of the T7-RelA proteins with antibodies to acetylated lysine confirmed dose-related increases in RelA acetylation by p300 in lanes 3 and 4 and dose-related deacetylation of RelA by HDAC3 in lanes 5 and 6 (*24*). These findings suggest that acetylation of RelA prevents IκBα binding, whereas deacetylation of RelA by HDAC3 stimulates IκBα binding.

To test whether the HDAC3-induced nuclear export of RelA is dependent on IκBα, we studied the subcellular localization of GFP-RelA in murine embryo fibroblasts (MEFs) isolated from wild-type or IκBα$^{-/-}$ mice produced by targeted gene disruption

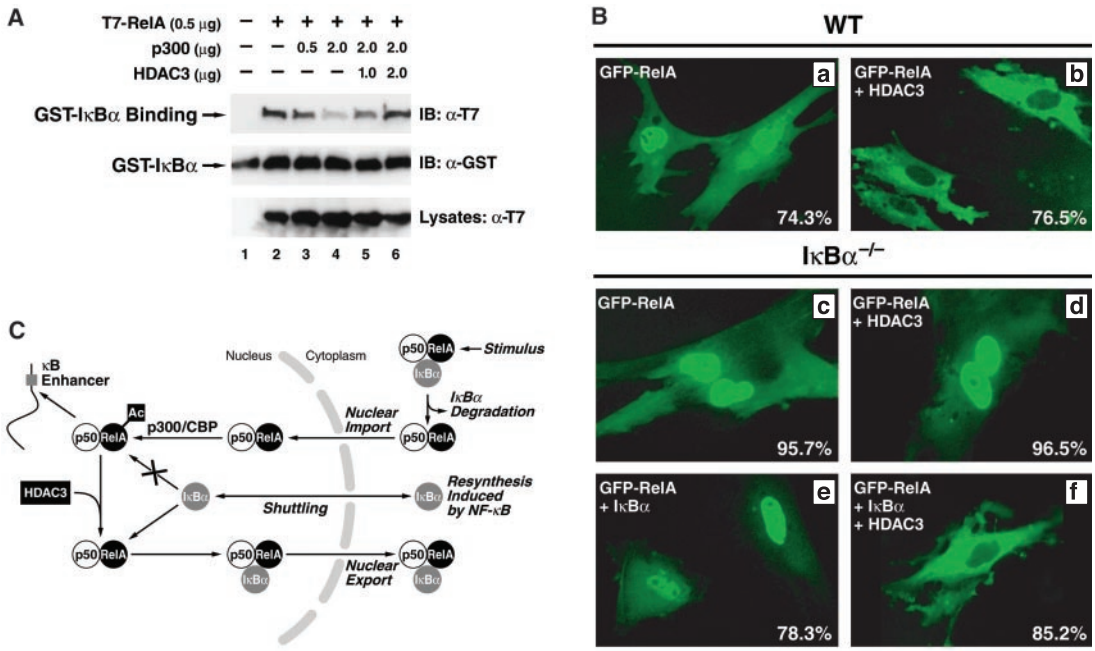

Fig. 4. (**A**) Deacetylated RelA displays greater IκBα-binding activity than acetylated RelA. 293T cells were cotransfected with T7-RelA and p300 in the presence or absence of HDAC3 expression vector DNA as indicated. Twenty-four hours later, 50 μl of each whole-cell lysate was incubated with GST-IκBα (1.0 μg). The levels of RelA captured by the GST-IκBα matrix under each condition were assessed by immunoblotting with anti-T7 (upper panel). The total amounts of GST-IκBα and RelA present in the reaction mixtures are shown in the lower two panels. (**B**) HDAC3 does not stimulate GFP-RelA nuclear export in IκBα-deficient MEFs. MEF cells from wild-type and IκBα$^{-/-}$ mice were transfected with GFP-RelA (0.3 μg) (panels a and c) or GFP-RelA (0.3 μg) and HDAC3 (1.7 μg) (panels b and d) expression vector DNA. The IκBα$^{-/-}$ MEFs were also reconstituted with limiting amounts of IκBα expression vector (0.1 μg) in the absence and presence of HDAC3 (panels e and f). Average percentages of cells displaying the depicted phenotype derived from inspection of at least 120 cells present in multiple microscopic fields from two independent experiments are shown in the lower right corner of each panel. (**C**) Schematic model for the role of HDAC3-mediated deacetylation of RelA as an intranuclear molecular switch promoting IκBα binding and IκBα-dependent nuclear export of the NF-κB complex. This deacetylation-controlled response both leads to the termination of the NF-κB transcriptional response and aids in reestablishing latent cytoplasmic forms of NF-κB bound to IκBα, thereby preparing the cell to respond to the next NF-κB–inducing stimulus.

(*25*) (Fig. 4B). In wild-type MEFs, GFP-RelA was principally expressed in the nucleus (panel a), whereas the coexpression of HDAC3 induced a cytoplasmic pattern of GFP-RelA epifluorescence (panel b). However, a very different pattern was obtained in the IκBα$^{-/-}$ MEFs. Whereas GFP-RelA also exhibited a nuclear pattern of epifluorescence, the coexpression of HDAC3 in these IκBα$^{-/-}$ MEFs failed to induce a cytoplasmic redistribution of RelA (panel d). Reconstitution of these IκBα$^{-/-}$ cells by transfection with small quantities of an IκBα expression vector restored HDAC3-induced cytoplasmic expression of the GFP-RelA protein (panel f). In the absence of HDAC3, GFP-RelA remained principally nuclear, indicating that the levels of IκBα expressed were not sufficient on their own to produce cytoplasmic sequestration of GFP-RelA in these IκBα$^{-/-}$ MEFs (panel e). These results indicate that IκBα is required for the nuclear export of deacetylated forms of RelA, which display increased binding of IκBα.

These findings reveal a new mechanism through which nuclear NF-κB function is regulated (Fig. 4C). RelA is subject to stimulus-coupled acetylation likely mediated through the p300 and CBP coactivators. One biological consequence of this modification is that acetylated RelA becomes a very poor substrate for binding by newly synthesized IκBα. Whether p50 or perhaps IκBα are similarly subject to biologically important acetylation/deacetylation reactions remains to be explored. Our studies identify acetylated RelA as a novel nonhistone substrate of HDAC3. As such, HDAC3-mediated deacetylation functions as an intranuclear molecular switch that when activated initiates a series of events culminating in the termination of the NF-κB transcriptional response. The IκBα-dependent nuclear export of the HDAC3-deacetylated RelA-containing complexes also serves to replenish the depleted cytoplasmic pool of latent NF-κB–IκBα complexes needed for the next inductive NF-κB response in these cells.

References and Notes

1. A. S. Baldwin Jr., *Annu. Rev. Immunol.* **14**, 649 (1996).
2. S. Ghosh, M. J. May, E. B. Kopp, *Annu. Rev. Immunol.* **16**, 225 (1998).
3. M. Karin, *Oncogene* **18**, 6867 (1999).
4. N. D. Perkins *et al.*, *Science* **275**, 523 (1997).
5. M. O. Hottiger, L. K. Felzien, G. J. Nabel, *EMBO J.* **17**, 3124 (1998).
6. K. A. Sheppard *et al.*, *Mol. Cell. Biol.* **19**, 6367 (1999).
7. H. Zhong, R. E. Voll, S. Ghosh, *Mol. Cell* **1**, 661 (1998).
8. A. Imhof, A. P. Wolffe, *Curr. Biol.* **8**, 422 (1998).
9. D. E. Sterner, S. L. Berger, *Microbiol. Mol. Biol. Rev.* **64**, 435 (2000).
10. M. T. Kouzarides, *EMBO J.* **19**, 1176 (2000).
11. H. Kuo, C. D. Allis, *Bioessays* **20**, 615 (1998).
12. W. D. Cress, E. Seto, *J. Cell Physiol.* **184**, 1 (2000).
13. H. H. Ng, A. Bird, *Trends Biochem. Sci.* **25**, 121 (2000).
14. S. C. Sun, P. A. Ganchi, D. W. Ballard, W. C. Greene, *Science* **259**, 1912 (1993).
15. K. Brown, S. Park, T. Kanno, G. Franzoso, U. Siebenlist, *Proc. Natl. Acad. Sci. U.S.A.* **90**, 2532 (1993).
16. A. A. Beg, T. S. Finco, P. V. Nantermet, A. S. Baldwin Jr., *Mol. Cell. Biol.* **13**, 3301 (1993).
17. F. Arenzana-Seisdedos *et al.*, *Mol. Cell. Biol.* **15**, 2689 (1995).
18. F. Arenzana-Seisdedos *et al.*, *J. Cell Sci.* **110**, 369 (1997).
19. Supplementary figures and information on experimental protocols are available on *Science* Online at www.sciencemag.org/cgi/content/full/293/5535/1653/DC1.
20. H. Bogerd, W. C. Greene, *J. Virol.* **67**, 2496 (1993).
21. E. Soutoglou, N. Katrakili, I. Talianidis, *Mol. Cell* **5**, 745 (2000).
22. C. Spilianakis, J. Papamatheakis, A. Kretsovali, *Mol. Cell. Biol.* **20**, 8489 (2000)
23. M. Yoshida, S. Horinouchi, *Ann. N. Y. Acad. Sci.* **886**, 23 (1999).
24. L.-F. Chen, W. C. Greene, unpublished data.
25. J. F. Klement *et al.*, *Mol. Cell. Biol.* **16**, 2341 (1996).

26. We thank R. Goodman (Vollum Institute) and Y. Nakatani (NIH) for reagents; J. F. Klement (Jefferson Medical College) for providing the IκBα$^{-/-}$ MEF cells; and A. O'Mahony for helping establish cultures of these cells. We also thank J. Carroll for assistance in the preparation of the figures and R. Givens for assistance in the preparation of the manuscript. Supported in part by funds provided by the J. David Gladstone Institutes, Pfizer, and the University of California, San Francisco–Gladstone Institute of Virology and Immunology Center for AIDS Research (P30MH59037).

8 May 2001; accepted 3 July 2001

Allosteric Activation of a Spring-Loaded Natriuretic Peptide Receptor Dimer by Hormone

Xiao-lin He, Dar-chone Chow, Monika M. Martick, K. Christopher Garcia*

Natriuretic peptides (NPs) are vasoactive cyclic-peptide hormones important in blood pressure regulation through interaction with natriuretic cell-surface receptors. We report the hormone-binding thermodynamics and crystal structures at 2.9 and 2.0 angstroms, respectively, of the extracellular domain of the unliganded human NP receptor (NPR-C) and its complex with CNP, a 22–amino acid NP. A single CNP molecule is bound in the interface of an NPR-C dimer, resulting in asymmetric interactions between the hormone and the symmetrically related receptors. Hormone binding induces a 20 angstrom closure between the membrane-proximal domains of the dimer. In each monomer, the opening of an interdomain cleft, which is tethered together by a linker peptide acting as a molecular spring, is likely a conserved allosteric trigger for intracellular signaling by the natriuretic receptor family.

The natriuretic peptides (NPs) are three homologous peptide hormones that play important roles in the maintenance of cardiovascular homeostasis, blood pressure, and body fluid regulation (i.e., natriuresis) (*1*). Collectively, these hormones function as an endogenous counterbalance to the renin-angiotensin/aldosterone system, as well as the hypothalamic/pituitary/adrenal axis. The three members of this family are atrial (ANP) and brain (BNP) natriuretic peptides, which are produced by the heart; and CNP, which is expressed in endothelial cells (*1*). ANP and BNP are thought to be the primary regulators of peripheral natriuretic activity; CNP is present mainly in the brain. ANP, BNP, and CNP are highly homologous (~70% identical) and share as a common motif a 17–amino acid loop formed by a disulfide bond (Fig. 1). The lack of defined structure(s) in solution and the questionable relevance of "lowest energy" solution conformations to the receptor-bound conformation of peptide hormones mean that assessing the bioactive conformations of these peptides remains a general problem (*2*).

The actions of the NPs are mediated by three homologous single-transmembrane, glycosylated cell-surface receptors (NPR-A, -B, and -C) (*3*–*5*). These receptors possess about 30% homologous extracellular ligand-binding domains (ECDs) (~450 amino acids) with conserved topologies but possess different downstream activation mechanisms. NPR-C is the most promiscuous of the receptors, binding to all NPs with high affinity, whereas NPR-A and NPR-B are more specific for ANP and CNP, respectively (*6*). In the cases of NPR-A and NPR-B, hormone binding to the ECDs results in the production of intracellular cyclic guanosine 3′,5′-monophosphate by a guanylyl-cyclase activity that resides in the intracellular domains (*5*, *7*). For NPR-C, which represents over 95% of NPR in vivo, ligand binding results in both internalization and degradation (i.e., clearance), as well as signaling by heterotrimeric GTP-