minogen, and potentially other fibrinolytic components, within human populations could represent a significant susceptibility factor for bacterial infection. Another virulence determinant was recently shown to form complexes with fibrinogen that induce vascular leakage, potentially enhancing the severity of GAS infection (*25*). These observations highlight the potential role of infectious disease as a critical force in the evolution of the hemostatic system and the unusual species specificity of many coagulation factor interactions.

References and Notes

1. E. Braunwald, *N. Engl. J. Med.* **334**, 51 (1996).
2. D. Schott *et al.*, *N. Engl. J. Med.* **339**, 1679 (1998).
3. T. H. Bugge, M. J. Flick, C. C. Daugherty, J. L. Degen, *Genes Dev.* **9**, 794 (1995).
4. V. A. Ploplis *et al.*, *Circulation* **92**, 2585 (1995).
5. A. F. Drew *et al.*, *Blood* **91**, 1616 (1998).
6. D. Collen, *Nature Med.* **4**, 279 (1998).
7. O. A. Sodeinde *et al.*, *Science* **258**, 1004 (1992).
8. J. L. Coleman *et al.*, *Cell* **89**, 1111 (1997).
9. K. Lahteenmaki, P. Kuusela, T. K. Korhonen, *FEMS Microbiol. Rev.* **25**, 531 (2001).
10. A. L. Bisno, D. L. Stevens, *N. Engl. J. Med.* **334**, 240 (1996).
11. H. E. McCoy, C. C. Broder, R. Lottenberg, *J. Infect. Dis.* **164**, 515 (1991).
12. U. Ringdahl *et al.*, *J. Biol. Chem.* **273**, 6424 (1998).
13. A. C. Wistedt *et al.*, *J. Biol. Chem.* **273**, 24420 (1998).
14. J. Khil *et al.*, *J. Infect. Dis.* **188**, 497 (2003).
15. I. P. Gladysheva, R. B. Turner, I. Y. Sazonova, L. Liu, G. L. Reed, *Proc. Natl. Acad. Sci. U.S.A.* **100**, 9168 (2003).
16. M. D. Boyle, R. Lottenberg, *Methods* **21**, 99 (2000).
17. H. R. Lijnen, B. van Hoef, V. Beelen, D. Collen, *Eur. J. Biochem.* **224**, 863 (1994).
18. Materials and methods are available as supporting material on *Science* Online.
19. Z. Li, V. A. Ploplis, E. L. French, M. D. Boyle, *J. Infect. Dis.* **179**, 907 (1999).
20. J. Cohen, *Nature* **420**, 885 (2002).
21. H. R. Lijnen, K. Okada, O. Matsuo, D. Collen, M. Dewerchin, *Blood* **93**, 2274 (1999).
22. A. Heath, V. J. DiRita, N. L. Barg, N. C. Engleberg, *Infect. Immun.* **67**, 5298 (1999).
23. C. McKillop, W. Edgar, C. D. Forbes, C. R. Prentice, *Nature* **255**, 638 (1975).
24. L. Johansson *et al.*, *Science* **301**, 373 (2003).
25. H. Herwald *et al.*, *Cell* **116**, 367 (2004).

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Supporting Online Material

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E Protein Silencing by the Leukemogenic AML1-ETO Fusion Protein

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The AML1-ETO fusion protein, generated by the t(8;21) chromosomal translocation, is causally involved in nearly 15% of acute myeloid leukemia (AML) cases. This study shows that AML1-ETO, as well as ETO, inhibits transcriptional activation by E proteins through stable interactions that preclude recruitment of p300/CREB-binding protein (CBP) coactivators. These interactions are mediated by a conserved ETO TAF4 homology domain and a 17–amino acid p300/CBP and ETO target motif within AD1 activation domains of E proteins. In t(8;21) leukemic cells, very stable interactions between AML1-ETO and E proteins underlie a t(8;21) translocation-specific silencing of E protein function through an aberrant cofactor exchange mechanism. These studies identify E proteins as AML1-ETO targets whose dysregulation may be important for t(8;21) leukemogenesis, as well as an E protein silencing mechanism that is distinct from that associated with differentiation-inhibitory proteins.

The t(8;21) chromosomal translocation fuses an N-terminal region of the AML1 transcription factor to a nearly complete ETO protein and is one of the most frequent chromosomal abnormalities seen in both childhood and adult acute myeloid leukemia (AML) (fig. S1) (*1*, *2*). ETO and *Drosophila* Nervy share four highly similar Nervy homology regions (NHR1, -2, -3, and -4). Both ETO and AML1-ETO associate with histone deacetylase (HDAC) complexes and, independently, form high–molecular-weight nuclear oligomers (*3*). The NHR1 region (also known as the TAF4 homology or TAFH domain) also displays significant homology with a conserved region of TBP-associated factor 4 (TAF4) proteins (fig. S1) that reside within the TFIID transcription factor (*1*, *2*).

Mass spectrometric analyses of anti-FLAG immunoprecipitates from nuclear extracts of FLAG-ETO–expressing HeLa cells identified two predominant ETO-interacting polypeptides (Fig. 1A, left), whose identities were determined to be MTGR1 (*4*), an ETO dimerization partner, and the basic helix-loop-helix (bHLH) transcription factor HEB (HeLa E-box–binding protein), a member of the E protein family that also includes E2A and E2-2 (*5*, *6*). Western blots revealed additional ETO associations with the E protein E2A and, consistent with published results (*3*), with multiple components of HDAC complexes (Fig. 1A, right). Analyses of FLAG-HEB immunoprecipitates (from transfected cells) revealed extremely stable ETO-HEB associations (resistant to up to 1 M NaCl and high concentrations of detergents) and further indicated that HEB directly interacts with ETO (or with AML1-ETO) in a stoichiometric manner (fig. S2, A and B).

Ectopic expression of ETO or AML1-ETO completely abolished transactivation by a Gal4-HEB fusion protein and, further, converted it to a potent repressor, whereas it only minimally affected the baseline transcription observed with Gal4-DBD (Fig. 1B) or transactivation observed with Gal4-VP16 or liganded Gal4-TR (fig. S2C). In further analyses with an E-box–containing template, ectopic ETO similarly abrogated transactivation by HEB (Fig. 1C, lane 2 versus lane 3) in a dose-dependent manner (fig. S3B). Next, we determined that an ETO TAFH (eTAFH) domain (fig. S3A) is both necessary and sufficient for inhibiting HEB-dependent transcription. Thus, removal of eTAFH domain residues 93 to 189 (Fig. 1C), but not other regions such as NHR2 (Fig. 1C), NHR3, and ZnF (fig. S3, A and B), completely abolished the inhibitory effect. Furthermore, the eTAFH domain alone (Fig. 1C), with a critical requirement for subregions 93 to 109 and 152 to 179 (fig. S3, A and B), showed a potent inhibition of HEB transcription, although to a lower magnitude than that effected by full-length ETO (see also figs. S3B and S4E). This suggests contributions from other ETO regions to total inhibition of HEB function. Consistent with their ability to inhibit HEB-dependent transcription, ectopic ETO and AML1-ETO, but not the eTAFH-deleted ETO mutant (ETOΔeTAFH), strongly interacted with HEB in vivo (Fig. 1D). Further analyses documented direct eTAFH-HEB inter-

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actions in solution (fig. S3C) and on an E-box element both in vitro (Fig. 1E) and in vivo (fig. S3D). Together, these results establish the eTAFH domain as the ETO region that interacts with HEB. Ectopic ETO and AML1-ETO similarly selectively target and inhibit activation by E proteins E2A and E2-2 [fig. S4, A to C, and (*7*)]. Moreover, consistent with a requirement for ubiquitous E proteins as cofactors for tissue-specific (class B) bHLH transcription factors such as MyoD (*8*), ETO markedly inhibited MyoD-dependent transactivation (fig. S4D). This inhibition requires both the eTAFH domain (fig. S4D) and another ETO region or regions (fig. S4E) that presumably help to overcome the MyoD activation domain through recruitment of HDAC complexes.

E proteins contain two conserved activation domains (an N-terminal AD1 and a central AD2) and a C-terminal bHLH-type DNA binding domain. We next determined that ETO selectively targets the AD1 activation domain. First, the AD1 domain of either HEB (residues 1 to 99) or E2A, as a Gal4 fusion, is sufficient both for activation and for ETO-targeted inhibition (Fig. 2A and fig. S5D). Second, whereas ectopic ETO effected a marked dose-dependent repression of AD1-mediated transcription, it minimally affected transcription (possibly attributed to AD2) associated with the AD1-deleted HEB mutant (ΔAD1, residues 100 to 682) (Fig. 2B). In accordance, ETO failed to interact with the ΔAD1 protein in vivo while showing a strong interaction with full-length HEB (Fig. 2C). Finally, the ETO-HEB interaction was recapitulated in solution with the two defined interaction domains eTAFH and AD1 (fig. S4F).

An alignment of E protein AD1 domains (fig. S5A) reveals a highly conserved region (corresponding to residues 11 to 27 of HEB) characteristic of LXXLL-containing (L, Leu; X, any amino acid) amphipathic helices implicated in protein-protein interactions (*9*). Indicative of a requirement of this region for both ETO interaction and transactivation by AD1, a single Leu^{17}→Ala^{17} (L17A) point mutation (fig. S5C) disrupted both physical (Fig. 2D, top) and functional (Fig. 2D, bottom; see also figure legend) interactions of AD1 with ETO, as well as AD1-elicited activation (Fig. 2D, bottom). A proteomic search for polypeptides differentially bound to AD1, but not to inactive AD1-L17A, identified p300 and CREB-binding protein (CBP) histone acetyl transferases (HATs) as major cofactors for AD1 (fig. S6A). Thus, AD1 but not the L17A mutant interacts specifically with p300 and CBP, but not with other factors such as GCN5, TFIID (monitored by TAF4 and TBP), and TFIIE (fig. S6B). Analyses of truncated AD1 derivatives revealed that the conserved 17-residue fragment, HEB(11-27), is sufficient both for activation and for ETO-dependent silencing (Fig. 3A). In accordance, HEB(11-27) displayed strong selective interactions both with p300/CBP and with ETO or eTAFH (Fig. 3B). We thus designated this motif as PCET (p300/CBP and ETO target in E proteins). The lack of PCET interactions with N- and C-terminal truncated eTAFH explains the inability of these mutants to inhibit HEB activation (Fig. 3B and fig. S3, A and B). Consistent with an essential role of PCET in mediating p300/CBP recruitment, further analyses of transfected components showed that AD1 is required for HEB association both with a HAT activity and with endogenous p300 in 293T cells (Fig. 3C, lanes 1, 2, and 4).

We next determined that ETO interaction with HEB through its eTAFH domain blocks p300/CBP recruitment by HEB. Thus, coexpression of ETO dramatically lowered the levels of both p300 and the HAT activity associated with ectopic FLAG-HEB in 293T cells (Fig. 3C, lane 2 versus lane 3). In accordance, neither p300 nor HAT activity was found to associate with FLAG-ETO–bound HEB protein (fig. S5B). Moreover, an in vitro analysis showed that eTAFH formed a stoichiometric complex with HEB and dramatically inhibited HEB interactions with p300/CBP but not with TFIID (Fig. 3D, lane 2 versus lane 3). Along with the observation that TFIID does not interact detectably with AD1 (fig. S6B), this indicates that HEB may independently recruit both p300/CBP HAT cofactors and TFIID components through distinct domains. Further analyses of PCET (fig. S5, C to E, and Fig. 3E) showed that whereas hydrophobic residues (green), such as the L17

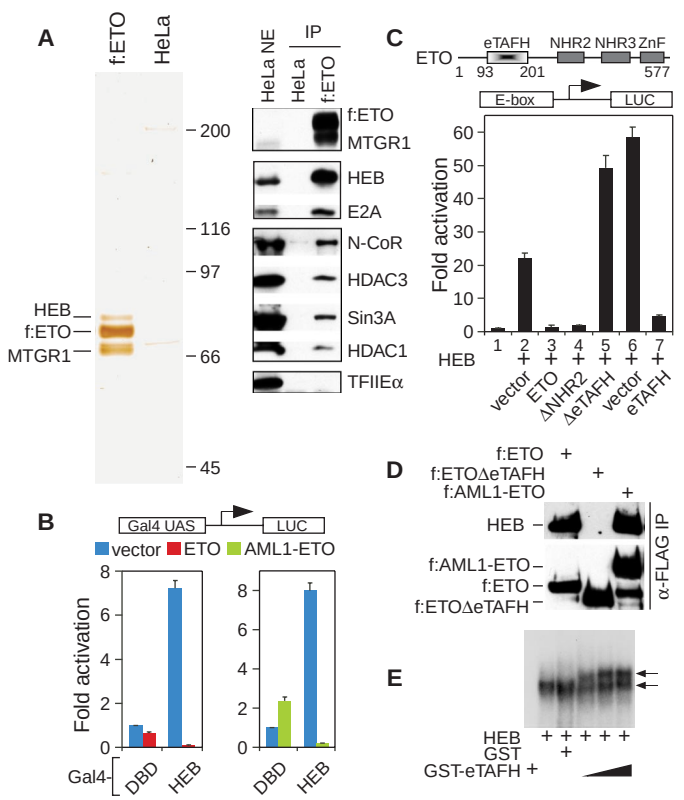

Fig. 1. ETO, through eTAFH, interacts with HEB and represses its transactivation function. (**A**) ETO interacts with HEB. (Left) Silver stain showing ETO-interacting polypeptides purified from FLAG-ETO–expressing cells. (Right) immunoblots of polypeptides purified as at left. (**B**) ETO and AML1-ETO inhibit HEB-dependent activation. (**C**) The eTAFH domain mediates inhibition of HEB-dependent activation. Luciferase assays in (B) and (C) used Gal4-based (B) or E-box–based (C) reporters and expression vectors as indicated at the bottom. (**D**) Requirement of eTAFH domain for ETO interactions with HEB in vivo, as revealed by immunoblot analysis of FLAG antibody immunoprecipitates (α-FLAG IP) from cells transfected with the indicated vectors (top). (**E**) eTAFH interacts with E-box–bound HEB, as revealed by electrophoretic mobility shift assay.

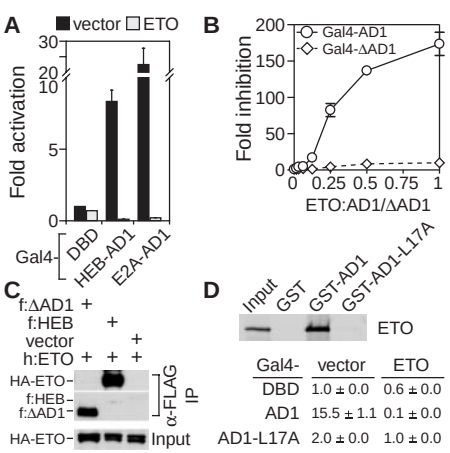

Fig. 2. ETO targets the E protein AD1 activation domain. (**A**) AD1 is sufficient for ETO-mediated inhibition. (**B**) AD1-independent HEB activity, unlike AD1 activity, is not inhibited by ETO. Luciferase assays in (A) and (B) were as in Fig. 1B, with the indicated vectors. (**C**) AD1 is required for HEB interaction with ETO in vivo (FLAG antibody coimmunoprecipitation/immunoblot assays were as in Fig. 1D). (**D**) An intact LXXLL motif is required for both physical [top, glutathione S-transferase (GST) pull-down assay] and functional (bottom, Gal4-based luciferase assay) interactions of AD1 with ETO as well as for maximal AD1-dependent activation. Note also the 155-fold (complete) inhibition of AD1 activity versus the twofold (incomplete) inhibition of the residual AD1-L17A activity.

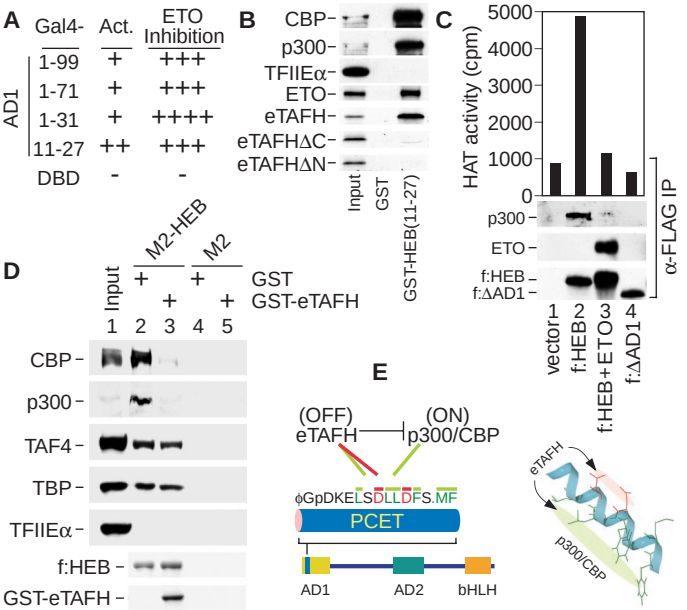

Fig. 3. ETO blocks p300/CBP recruitment by HEB. (**A**) The HEB(11-27) fragment is sufficient both for activation and for ETO-mediated inhibition. Ranges for fold activation and fold inhibition (assayed as in Fig. 2A) are as follows: +, 5 to 20; ++, 30 to 50; +++, 60 to 150; and ++++, >250. (**B**) PCET interacts with both p300/CBP and ETO or eTAFH. p300, CBP, and TFIIEα were from HeLa nuclear extracts; ETO and eTAFH and derivatives were translated in vitro. eTAFHΔC, residues 93 to 151; eTAFHΔN, residues 110 to 201. (**C**) ETO blocks AD1-dependent HEB interactions both with p300 and with HAT activity. FLAG antibody immunoprecipitates from cells transfected with indicated vectors (bottom) were analyzed by immunoblot (center) and for HAT activity (top). (**D**) Immunoblot analyses (top five panels) and Coomassie blue stain (bottom two panels) of polypeptides from HeLa extracts bound to M2-agarose–immobilized FLAG-HEB in the presence of GST or GST-eTAFH. (**E**) PCET functions as a control switch for E protein function. eTAFH recognizes distinct residues (red) in addition to residues (green) jointly recognized by eTAFH and p300/CBP. φ, Ile, Leu, or Val; p, Ser or Thr. The α-helical model of PCET at right shows distinct locations of at least two surfaces involved in eTAFH and p300/CBP interactions.

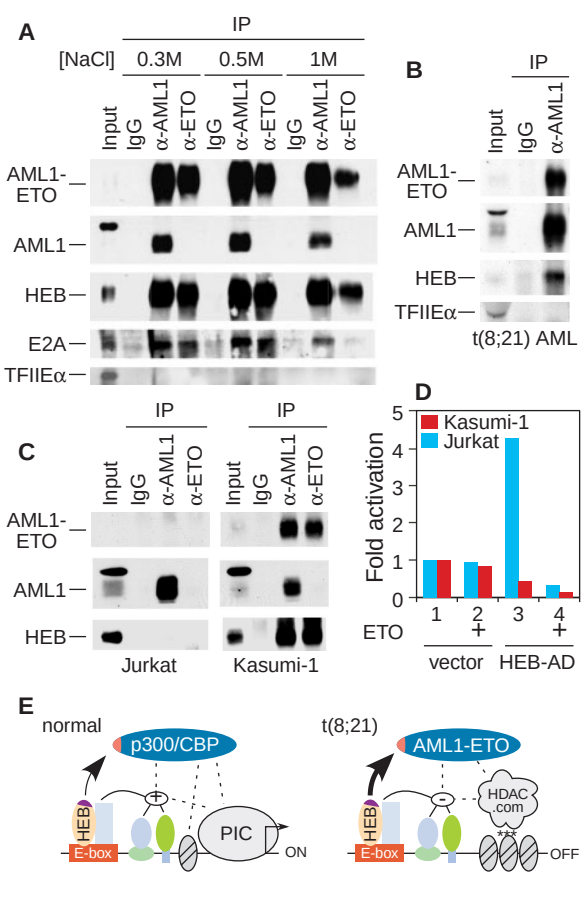

Fig. 4. AML1-ETO stably interacts with E proteins in t(8;21) leukemic cells. (**A** and **B**) Highly stable associations of endogenous E proteins with endogenous AML1-ETO in both Kasumi-1 (A) and primary t(8;21) leukemic (B) cells. (**C**) HEB interactions with ETO-containing polypeptide(s) are specific to t(8;21) cells. In (A) to (C), immunoprecipitates with indicated antibodies (top) were analyzed by immunoblot. (**D**) Suppression of ectopic HEB-dependent activity is specific to t(8;21) cells. Gal4-HEB AD was assayed in a Gal4-based Luciferase reporter assay in the presence and absence of ectopic ETO. (**E**) Model for AML1-ETO–driven aberrant cofactor exchange on E-box–bound E proteins such as HEB, and consequent E protein silencing in t(8;21) cells. Dashed lines indicate physical/functional interactions among cofactors or factors. eTAFH (and an equivalent region in p300/CBP) and PCET are shown in red and purple, respectively. Nucleosomes are shown as hatched gray circles. Promoter bound activators are shown as colored objects bound to DNA. HDAC.com, HDAC-containing complexes; pic, preinitiation complex.

described earlier, are important for recognition by both ETO and p300/CBP, adjacent Asp residues (red) provide additional separate surfaces specific for ETO (Fig. 3E, right). The differential recognition of these negatively charged residues may result in a greater affinity for ETO and consequently provides a structural basis for a dominant, and possibly irreversible, block of p300/CBP interactions by ETO.

We next explored the physiological relevance of these interactions to t(8;21) leukemogenesis by examining endogenous factors in t(8;21) leukemic cells. Kasumi-1 cells carry a t(8;21) translocation and express a high level of the AML1-ETO fusion protein but fail to express any detectable ETO protein (fig. S7A). Similar results were observed for another t(8;21) leukemic cell line (SKNO-1) (*7*). These data are consistent with earlier observations that ETO is not expressed in normal hematopoietic cells (*10*, *11*), and they further suggest that an ETO-associated activity could potentially contribute to t(8;21) leukemogenesis as a result of the aberrant high level of expression of AML1-ETO. In agreement with data presented above, endogenous HEB and E2A proteins were found in a very stable (resistant to 1% Triton X-100 and up to 1 M NaCl) natural complex(s) with AML1-ETO in Kasumi-1 cells (Fig. 4A). Similar analyses of SKNO-1 cells (fig. S7B) and of primary hematopoietic cells from a t(8;21) AML patient (Fig. 4B) further suggested that formation of a stable E protein:AML1-ETO complex is probably a general feature of t(8;21) cells. A control analysis failed to detect such interactions in a T lymphocyte cell line (Jurkat) (Fig. 4C) that does not express AML1-ETO (Fig. 4C) or detectable ETO (*7*). Thus, associations of E proteins, such as HEB, with an ETO-containing polypeptide in hematopoietic cells are probably dependent on the t(8;21) translocation and the consequent high level of expression of AML1-ETO.

As observed with ectopic ETO expression, a high level of expression of endogenous AML1-ETO in Kasumi-1 cells dominantly blocks p300 association with HEB and recruits HDACs to its AD1 domain (fig. S7, C and D, and supporting online text). These results clearly point to an AML1-ETO–dependent aberrant cofactor exchange for HEB, and likely other E proteins, in t(8;21) cells. Further supporting this idea, a Gal4-HEB activation domain (residues 1 to 548) fusion protein displayed an activation function in Jurkat cells, which do not express AML1-ETO, but not in Kasumi-1 cells, where a repression function is evident (Fig. 4D, lanes 1 and 3). This reflects an ETO-associated activity because ectopic ETO enhanced HEB silencing in Kasumi-1 cells and converted HEB into a repressor in Jurkat cells (Fig. 4D, lanes 3 and 4). Thus, an apparent consequence of the t(8;21) translocation is to allow a high level of expression of an ETO-containing polypeptide that is otherwise not expressed in normal hematopoietic cells (*10*,

11). Accordingly, in normal hematopoietic precursors (Fig. 4E, left), expression of essential (yet to be identified) genes for proper intracellular pathways [such as those involved in important checkpoint controls (*5*, *12*)] may be positively regulated by HEB (or other E proteins) through its promoter interactions with E-box elements (either as homodimers or as heterodimers with cognate partners), through its associations with p300/CBP HATs, and through the resulting cooperative interactions with adjacent promoter-bound activators. In contrast, in t(8;21) cells (Fig. 4E, right), expression of these genes may be silenced because of a dominant interaction of HEB with AML1-ETO that precludes promoter occupancy by p300/CBP but facilitates occupancy by HDAC-containing complexes. Inhibition of these gene expression events may thus predispose cells to further leukemogenic events, possibly as a result of dysregulated checkpoint control.

Beyond defining E proteins as AML1-ETO/ETO targets, our studies also elucidate an E protein silencing mechanism that is fundamentally different from that associated with Id proteins (inhibitors of DNA binding/differentiation) (*13*). Thus, although Id interactions with DNA binding regions of E proteins passively block corresponding promoter interactions, ETO/AML1-ETO interactions with AD1 of promoter-bound E proteins effect a silencing by directing an exchange of cofactors (HATs versus HDACs) that are recruited to target promoters. Like ETO, ETO-related proteins MTGR1 and ETO-2 similarly interact with and inhibit the function of E proteins (*7*). This mechanism may underlie a previously described context-dependent repressive function of the E protein AD1 domain and an enhancer-specific E protein activity (*14*).

E proteins (class A bHLH proteins) are ubiquitously expressed transcription factors that play key roles in the regulation of cell growth and differentiation and programmed cell death (*5*, *6*, *8*, *15*, *16*). E2A is essential for early B cell differentiation events and is a potential tumor suppressor (*6*, *15*). HEB has been implicated in both myogenesis and hematopoiesis (*5*, *17*). Fusions involving E2A (*5*) and HEB (*18*) AD1 domains are associated with leukemogenesis or tumorigenesis. Moreover, inhibition of E protein function by Id proteins negatively regulates cell differentiation and induces proliferation (*13*), an event whose dysregulation is often associated with oncogenesis. Similarly, and consistent with dysregulation of E protein functions by AML1-ETO, it has been shown that AML1-ETO directly induces aberrant hematopoietic cell proliferation (*19*), promotes extensive expansion and self-renewal of human hematopoietic stem cells (*20*–*22*) (the physiological target of many acute myeloid leukemias), and inhibits maturation of multiple lymphohematopoietic lineages (*23*), but is by itself insufficient for leukemogenesis (*24*). These observations further strengthen the idea that E proteins are major physiological targets of AML1-ETO in t(8;21) leukemogenic cells. Our results lead to the hypothesis that there are E protein target genes whose dysregulation by AML1-ETO may be important for t(8;21) leukemogenesis, and they set the stage for identification of these genes and for analyses of the structural basis of the underlying, newly defined regulatory factor interactions.

References and Notes

1. J. D. Licht, *Oncogene* **20**, 5660 (2001).
2. J. M. Scandura, P. Boccuni, J. Cammenga, S. D. Nimer, *Oncogene* **21**, 3422 (2002).
3. B. A. Hug, M. A. Lazar, *Oncogene* **23**, 4270 (2004).
4. I. Kitabayashi *et al.*, *Mol. Cell. Biol.* **18**, 846 (1998).
5. M. W. Quong, W. J. Romanow, C. Murre, *Annu. Rev. Immunol.* **20**, 301 (2002).
6. M. E. Massari, C. Murre, *Mol. Cell. Biol.* **20**, 429 (2000).
7. J. Zhang, R. G. Roeder, unpublished data.
8. A. B. Lassar *et al.*, *Cell* **66**, 305 (1991).
9. D. M. Heery, E. Kalkhoven, S. Hoare, M. G. Parker, *Nature* **387**, 733 (1997).
10. H. Miyoshi *et al.*, *EMBO J.* **12**, 2715 (1993).
11. F. Calabi, R. Pannell, G. Pavloska, *Mol. Cell. Biol.* **21**, 5658 (2001).
12. I. Engel, C. Murre, *EMBO J.* **23**, 202 (2004).
13. M. B. Ruzinova, R. Benezra, *Trends Cell Biol.* **13**, 410 (2003).
14. M. Markus, Z. Du, R. Benezra, *J. Biol. Chem.* **277**, 6469 (2002).
15. I. Engel, C. Murre, *Proc. Natl. Acad. Sci. U.S.A.* **96**, 996 (1999).
16. A. Pagliuca, P. Gallo, P. De Luca, L. Lania, *Cancer Res.* **60**, 1376 (2000).
17. J. S. Hu, E. N. Olson, R. E. Kingston, *Mol. Cell. Biol.* **12**, 1031 (1992).
18. H. Sjogren *et al.*, *Cancer Res.* **60**, 6832 (2000).
19. T. Okuda *et al.*, *Blood* **91**, 3134 (1998).
20. B. A. Hug, S. Y. Lee, E. L. Kinsler, J. Zhang, M. A. Lazar, *Cancer Res.* **62**, 2906 (2002).
21. J. C. Mulloy *et al.*, *Blood* **99**, 15 (2002).
22. C. G. de Guzman *et al.*, *Mol. Cell. Biol.* **22**, 5506 (2002).
23. M. Schwieger *et al.*, *J. Exp. Med.* **196**, 1227 (2002).
24. K. L. Rhoades *et al.*, *Blood* **96**, 2108 (2000).

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Supporting Online Material

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Small Interfering RNA–Induced Transcriptional Gene Silencing in Human Cells

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Small interfering RNA (siRNA) and microRNA silence genes at the transcriptional, posttranscriptional, and/or translational level. Using human tissue culture cells, we show that promoter-directed siRNA inhibits transcription of an integrated, proviral, elongation factor 1 alpha (EF1A) promoter–green fluorescent protein reporter gene and of endogenous EF1A. Silencing was associated with DNA methylation of the targeted sequence, and it required either active transport of siRNA into the nucleus or permeabilization of the nuclear envelope by lentiviral transduction. These results demonstrate that siRNA-directed transcriptional silencing is conserved in mammals, providing a means to inhibit mammalian gene function.

Small 21- to 25-nucleotide RNAs have diverse biological roles in eukaryotes, including transposon silencing and antiviral defense by small interfering RNAs (siRNAs) and developmental gene regulation by microRNAs (miRNAs) (*1*–*3*). siRNAs and miRNAs are processed from double-stranded precursors by the ribonuclease (RNase) III–RNA helicase Dicer (*1*). Argonaute proteins can bind small RNAs and are components of effector complexes that downregulate gene expression by several mechanisms (*4*). Small RNAs with perfect homology to their target can cause specific mRNA cleavage (called RNA interference), whereas those with mismatches to their target mediate translational inhibition (*3*). Small RNA–mediated transcriptional gene silencing was first observed in plants through the use of inverted-

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