Published in final edited form as:

Cancer Lett. 2010 May 28; 291(2): 237–245. doi:10.1016/j.canlet.2009.10.018.

Histone acetyltransferase p300 is a coactivator for transcription factor REL and is C-terminally truncated in the human diffuse large B-cell lymphoma cell line RC-K8

Michael R. Garbati, Gökçen Alço, and Thomas D. Gilmore*

Department of Biology, Boston University, Boston, MA 02215, USA

Abstract

Human c-Rel (REL) is a member of the NF- κ B family of transcription factors. REL's normal physiological role is in the regulation of B-cell proliferation and survival. The *REL* gene is amplified in many human B-cell lymphomas and overexpression of REL can transform chicken lymphoid cells. In this report, histone acetyltransferase p300 enhanced REL-induced transactivation and interacted with REL both in vitro and in REL-transformed chicken spleen cells and the B-lymphoma cell line RC-K8, in which REL is constitutively active and required for proliferation. However, due to a deletion in the *EP300* locus, only a C-terminally truncated form of p300 is expressed in RC-K8 cells. These results suggest a role for p300 in REL-mediated oncogenic activity in B-lymphoma.

Keywords

c-Rel; p300; CBP; RC-K8; Diffuse large B-cell lymphoma

1. Introduction

The human *c-rel* proto-oncogene (*REL*) encodes a transcription factor in the NF- κ B family of proteins. Expression of *c-rel* is important for normal and malignant B-cell proliferation and survival [1]. *c-rel* knockout mice have immune deficiencies because their B cells do not proliferate in response to mitogenic stimulation [2], and misregulation of REL has been implicated in B-cell malignancy in several ways. The *REL* gene is amplified or mutated in some types of B-cell lymphoma, including diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, and Hodgkin's lymphoma [1,3]. Overexpression of REL can transform chicken lymphoid cells [4]. Additionally, REL is constitutively active in many B-lymphoma cell lines, including the human DLBCL cell line RC-K8 [5].

The REL protein contains a conserved N-terminal DNA-binding/dimerization domain of approximately 300 amino acids (aa) called the Rel homology domain (RHD). The C-terminal half of REL (aa 296-587) contains a transactivation domain (TAD) with two subdomains:

*Corresponding author: T.D. Gilmore, Biology Department, Boston University, 5 Cummington Street, Boston, MA 02215, USA; 617-353-5444 (phone); 617-353-6340 (fax); gilmore@bu.edu.

Conflict of interest statement

No conflict of interest is declared.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

TAD1 consists of aa 422-497 and TAD2 aa 518-587 [6,7]. Deletion of the entire C-terminal TAD in mice abolishes the ability of c-Rel to perform its normal functions in the B-cell immune response [8]. Moreover, the transforming activity of REL requires at least one of its two TAD subdomains to be present [7]. Therefore, understanding the mechanism by which REL activates transcription will help elucidate REL's normal and oncogenic activities.

The related histone acetyltransferases CBP and p300 can act as coactivators for many transcription factors [9,10]. Several groups have shown that CBP/p300 can interact with NF- κ B transcription factor RelA to enhance its transactivating activity [11–19], but little is known about the effect of CBP/p300 on other NF- κ B transcription factors. Co-expression of p300 has been shown to enhance the transactivation ability of REL [20,21], and Wang et al. [19] showed a weak interaction between REL and CBP in whole cell extracts. However, a direct and functional nuclear interaction between REL and CBP/p300 has not been rigorously described. In this report, we show that both p300 and CBP can interact with REL through its C-terminal TAD sequences, and that this interaction enhances REL-dependent transactivation. Additionally, we show that the RC-K8 DLBCL cell line expresses only a C-terminally truncated form of p300, which can still interact with nuclear REL.

2. Materials and methods

2.1. Plasmids

DNA manipulations were carried out by standard methods [22]. Complete details of all subclones and primers used in this study are described in supplementary information and at www.nf-kb.org.

2.2. Cell culture and transfection

A293 human embryonic kidney cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Biologos, Montgomery, IL, USA) as described [23]. RC-K8, BJAB, BL41, Daudi, IB4, and SUDHL4 human lymphoma cell lines, and REL-transformed chicken spleen cells were maintained in DMEM supplemented with 20% heat-inactivated FBS. The REL-transformed chicken spleen cell culture was generated using spleen necrosis virus vector JD-REL as described previously [4].

For transfections, A293 cells were seeded such that they were approximately 60% confluent on the following day when transfections were performed using polyethylenimine (PEI) (Polysciences, Warrington, PA, USA). On the day of transfection, DNA:PEI was incubated at a ratio of 1:3 in serum-free DMEM (200l for a 35-mm plate; 500 μ l for a 100-mm plate) for 15 min at room temperature. The DNA/PEI mixture was then added to two (35-mm plate) or ten ml (100-mm plate) of DMEM containing 10% FBS and the final mixture was added to the cells. The next day, the transfection media was replaced with fresh DMEM containing 10% FBS. Cells were harvested and lysed 24 h later.

2.3. GST pulldown assays

GST and GST-REL fusion proteins were expressed in *E. coli* and purified on glutathione-agarose beads as previously described [24]. Five percent of the protein-bound beads from each sample were separated on an SDS-polyacrylamide gel and stained with Coomassie Blue to verify that approximately equal amounts of each GST-fusion protein had been purified. The remaining beads were incubated with 3 mg of A293 or RC-K8 whole cell extract for 2 h at 4°C. The beads were then washed with AT lysis buffer (20 mM HEPES pH 7.9, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 20% v/v glycerol, 1% v/v Triton X-100, 20 mM NaF, 1 mM Na₄P₂O₇·10H₂O, 1 mM DTT, 1 mM Na₃VO₄, 1 mM phenylmethylsulfonylfluoride [PMSF],

1 µg/ml pepstatin, 1 µg/ml leupeptin, 10 µg/ml aprotinin [Sigma, St. Louis, MO, USA]) and proteins were eluted by heating at 95°C in SDS sample buffer. Proteins were electrophoresed on a 6% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane using a large-protein transfer buffer (20 mM Tris, 150 mM glycine, 0.05% SDS, 10% methanol). One percent of the amount of extract used for one pulldown (30 µg) was included on the gel as an input lane. The membrane was then subjected to anti-p300 or anti-CBP Western blotting.

2.4. Western blotting

Western blotting was performed as described previously [7,23]. The following antisera were used: rabbit anti-REL (1:10,000 dilution; anti-C-terminal, #265, Nancy Rice, NCI, Frederick, MD, USA), rabbit anti-REL (1:2500; anti-NLS, #1206, Nancy Rice), mouse anti-CBP (1:200; sc-7300, Santa Cruz Biotechnology, Santa Cruz, CA USA), rabbit anti-CBP (1:1000; #4772, Cell Signaling Technology, Danvers, MA, USA), rabbit anti-p300 (1:200; anti-N-terminal, sc-584, Santa Cruz Biotechnology), rabbit anti-p300 (1:200; anti-C-terminal, sc-585, Santa Cruz Biotechnology), rabbit anti-FLAG (1:1000; #2368, Cell Signaling Technology), rabbit anti-HA (1:500; sc-805, Santa Cruz Biotechnology), mouse anti-MYC (1:200; sc-40, Santa Cruz Biotechnology), rabbit anti-IκBα (1:500; sc-371, Santa Cruz Biotechnology), and rabbit β-tubulin (1:500; sc-9104, Santa Cruz Biotechnology).

2.5. Immunoprecipitation

For co-immunoprecipitation of overexpressed proteins, A293 cells in 100-mm plates were co-transfected with 5 µg HA-p300 and 5 µg FLAG-REL, FLAG-REL TAD1, FLAG-REL TAD2, FLAG-REL-RHD, or pcDNA-FLAG; or with 5 µg pCMV-CBP and 5 µg MYC-REL, MYC-REL-RHD, or pcDNA. Two days later, cells were washed in PBS and nuclear extracts were prepared as described previously [25]. For anti-FLAG immunoprecipitation, 500 µg of each nuclear extract was incubated with anti-FLAG M2 affinity gel (Sigma) in high salt buffer (20 mM HEPES pH 7.9, 400 mM NaCl, 1 mM EDTA, 1 mM EGTA) overnight at 4°C. For anti-MYC immunoprecipitations, 3 µg of anti-MYC antiserum (sc-40, Santa Cruz Biotechnology) was incubated with protein G Sepharose beads in high salt buffer overnight at 4°C. Antibody-bound beads were then washed and incubated with 500 µg of each nuclear extract in high salt buffer overnight at 4°C. Beads were then washed in high salt buffer and proteins were eluted by heating at 95°C in SDS sample buffer. Proteins were electrophoresed on a 6% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane using a large-protein transfer buffer as described above. For all samples, 1% of the amount of nuclear extract used for one pulldown (5 µg) was included on the gel as an input lane. The membranes were then subjected to anti-HA (to detect p300) or anti-CBP Western blotting.

Co-immunoprecipitations of endogenous REL with p300 or CBP were performed using the Nuclear Complex Co-IP Kit essentially as described by the manufacturer (Active Motif, Carlsbad, CA, USA). Three µg of normal rabbit IgG (sc-2027, Santa Cruz Biotechnology), anti-p300 antiserum (sc-584, Santa Cruz Biotechnology) or anti-CBP antiserum (#4774, Cell Signaling Technology) was incubated with protein A Sepharose beads (Amersham Biosciences, Piscataway, NJ, USA) in IP Low Buffer (Active Motif) overnight at 4°C. Antibody-bound beads were then washed with IP Low Buffer and incubated with 250 µg of nuclear extract from RC-K8 cells or REL-transformed chicken spleen cells in IP Low Buffer overnight at 4°C. Beads were washed with IP Low Buffer and proteins were eluted by heating at 95°C in SDS sample buffer. To detect REL and p300ΔC, proteins were electrophoresed on a 7.5% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane. To detect p300 or CBP, proteins were electrophoresed on a 6% SDS-polyacrylamide gel and transferred to a nitrocellulose membrane using a large-protein transfer buffer containing 0.05% SDS and 10% MeOH. One percent (for anti-REL) or 10% (for anti-p300 or anti-CBP) input lanes were

included. The membranes were then subjected to anti-REL, anti-p300, or anti-CBP Western blotting.

2.6. Luciferase reporter assays

Luciferase reporter assays were performed as described previously [24]. A293 cells in 35-mm plates were transfected with 0.5 μ g of reporter plasmid pGL2-3 $\times$ - κ B-luciferase and 0.5 μ g of normalization plasmid RSV- β gal. Cells were co-transfected with 0.5 μ g of pcDNA-REL or vector alone, along with 0.5 μ g of HA-p300, pCMV-CBP, or vector alone. Cells were harvested in Reporter Lysis Buffer (Promega, Madison, WI, USA) and luciferase activity was measured using the Luciferase Assay System (Promega). Luciferase and β -galactosidase activities were determined, and values were normalized to the relevant vector control (1.0). Values are the averages of three experiments performed with triplicate samples.

2.7. Northern blotting

Northern blotting was performed essentially as described previously [22]. Total RNA was isolated from A293 and RC-K8 cells using TRIzol Reagent according to the manufacturer's protocol (Invitrogen, Carlsbad, CA, USA). RNA was separated by agarose gel electrophoresis and then transferred by capillary action to a Zeta-Probe GT membrane (Bio-Rad, Hercules, CA, USA). RNA was UV-crosslinked to the membrane with a Stratalinker 2400 (Stratagene, La Jolla, CA, USA). Probes were produced using PCR products generated from the 5' approximately 500 bases of *EP300* (using primers p300-5'probe-For and p300-5'probe-Rev) and from approximately 600 bases of exon 31 of *EP300* (using primers p300-exon31-For2 and p300-exon31-Rev2) (see Fig. S1 and supplementary information). These PCR products were used as templates to generate random-primed 32 P-labeled DNA probes as previously described [26]. Membranes were prehybridized for 1 h in Amersham Rapid-hyb buffer (GE Healthcare, Little Chalfont, UK) at 65°C with rotation in a Robbins Model 2000 Micro Hybridization Incubator (Robbins Scientific, Sunnyvale, CA, USA). The denatured probe was then added to the hybridization buffer and incubated with the membrane overnight at 65°C with rotation. Membranes were washed twice with 50 ml of 2X SSC/0.1% SDS for 20 min each at 25°C, twice with 50 ml of 0.2X SSC/0.1% SDS for 15 min each at 65°C, and twice with 50 ml of 0.1X SSC/0.1% SDS for 15 min each at 65°C. Membranes were then exposed to X-ray film with intensifying screens for 2–7 days at –80°C.

2.8. Real-time PCR

For reverse transcription real-time quantitative PCR, total RNA was obtained from A293 and RC-K8 cells using TRIzol Reagent according to the manufacturer's protocol (Invitrogen). RNA was reverse transcribed into cDNA using M-MLV reverse transcriptase (Promega) and random primers (Promega). One thirtieth of the synthesized cDNA was combined with *EP300* primers (see Fig. S1) or *GAPDH* primers and Power SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA). PCRs were performed in the ABI Prism 7900HT Sequence Detection System (Applied Biosystems) using 40 cycles of 94°C for 15 sec and 60°C for 1 min. Cycle threshold (C_t) levels were obtained for each sample and the average of the three wells was calculated. To normalize the *EP300* samples, the C_t value for *GAPDH* cDNA amplification was subtracted from each value (ΔC_t). Then, the ΔC_t value for A293-cell samples was subtracted from each value for RC-K8-cell samples ($\Delta\Delta C_t$). Relative *EP300* mRNA expression levels were then calculated from the $\Delta\Delta C_t$ values ($2^{-\Delta\Delta C_t}$) as described previously [27]. Normalized relative expression values are the averages of three experiments and standard error was calculated.

For genomic qPCR, genomic DNA was isolated from BJAB and RC-K8 cells. Fifty ng of DNA was combined with *EP300* exon-specific forward and reverse primers and Power SYBR Green PCR Master Mix (Applied Biosystems). PCR was then performed as described above. The

C_t value for *EP300* exon 2 amplification was subtracted from each value (ΔC_t). Next, the ΔC_t value for each BJAB cell sample was subtracted from the value for the corresponding *EP300* exon from the RC-K8 cell sample ($\Delta\Delta C_t$). The relative abundance of each exon was then calculated from the $\Delta\Delta C_t$ values ($2^{-\Delta\Delta C_t}$) as described previously [27]. Normalized relative abundance values are the averages of three experiments and standard error was calculated. The sequences of all primers are listed in supplementary information.

2.9. Semi-quantitative RT-PCR

Total RNA was isolated from A293 and RC-K8 cells using TRIzol Reagent and reverse transcribed into cDNA as described above. Twenty-seven cycles of PCR was performed on 1/15 of the synthesized cDNA using *EP300* primers (see Fig. S1). PCR products were separated by agarose gel electrophoresis and visualized by ethidium bromide staining. ImageJ software (National Institutes of Health, Bethesda, MD, USA) was used to measure band densities. The value from the no-template control (NTC) lane was subtracted from the value for each band and then values were normalized to those of amplicons from cDNA obtained from A293 cells (1.0).

3. Results

3.1. CBP and p300 interacted with REL transactivation domain sequences in vitro

To determine whether CBP and p300 can interact with REL, pulldown experiments were performed using bacterially-expressed GST-fusion proteins containing subdomains of REL and A293 human embryonic kidney whole cell extracts (Fig. 1A). First, GST fusion proteins on beads were incubated with equal portions of an A293 cell extract, and the presence of CBP in the pulldowns was assessed by Western blotting. Fig. 1B shows that the REL transactivation domain (GST-REL-TAD) interacted strongly with CBP, the RHD (GST-REL-RHD) interacted weakly with CBP, and GST alone did not interact with CBP. To define the C-terminal REL sequences that interacted with CBP, GST-REL fusions containing deletions of one (GST-REL Δ TAD1; GST-REL Δ TAD2) or both (GST-REL Δ TAD1,2) of the C-terminal TAD subdomains were used in a similar pulldown experiment. GST-REL Δ TAD1 and GST-REL Δ TAD2 pulled down CBP, albeit less efficiently than the complete TAD in GST-REL-TAD (Fig. 1C). GST-REL Δ TAD1,2 and GST alone did not pull down REL. Taken together, these results indicate that each TAD subdomain contributed to REL's interaction with CBP.

To determine whether REL can also interact with p300, a similar pulldown experiment was performed. GST-REL-TAD interacted strongly with p300, GST-REL-RHD interacted weakly with p300, whereas GST-REL Δ TAD1,2 and GST alone did not interact with p300 (Fig. 1D). This indicates that p300, like CBP, bound to the REL TAD.

3.2. p300 and CBP interacted with REL in vivo and acted as coactivators for REL-induced transactivation

To determine whether REL interacts with p300 in vivo, FLAG-REL expression constructs for full-length REL, REL-RHD, REL Δ TAD1, and REL Δ TAD2 or vector alone were co-transfected with an HA-p300 expression plasmid into A293 cells. Nuclear extracts were later immunoprecipitated with anti-FLAG antiserum and immune complexes were subjected to anti-HA Western blotting. HA-p300 co-precipitated with full-length FLAG-REL, FLAG-REL Δ TAD1, and FLAG-REL Δ TAD2, but HA-p300 was not detected in immunoprecipitates from cells transfected with FLAG-REL-RHD or vector alone (Fig. 2A). These results suggest that p300 interacted with REL in vivo primarily through the C-terminal TAD of REL. To determine whether REL interacts with CBP in vivo, expression vectors for MYC-tagged versions of full-length REL, REL-RHD (lacking the entire REL TAD), or vector alone were co-transfected into A293 cells with a CBP expression vector. Nuclear extracts were

immunoprecipitated with anti-MYC antiserum, and immune complexes were subjected to anti-CBP Western blotting. CBP interacted with full-length MYC-REL, but interacted only weakly with MYC-REL-RHD (Fig. 2B). Therefore, similar to p300, CBP interacted with REL primarily through the C-terminal TAD of REL.

To determine whether p300 can affect REL-dependent transactivation, A293 cells were transfected with expression vectors for REL plus p300 or vector alone and a luciferase reporter plasmid with a promoter containing three upstream κ B sites. p300 enhanced REL-mediated transactivation by approximately two-fold (Fig. 2C, left panel). A similar experiment showed that CBP also enhanced REL activity by approximately two-fold (Fig. 2C, right panel).

3.3. RC-K8 cells express a C-terminally truncated p300 protein

In the course of screening human B-lymphoma cells for p300 expression, we discovered that the RC-K8 cell line expressed a smaller version of p300. That is, Western blotting of RC-K8 cell extracts using an antibody directed against the N terminus of p300 detected only one anti-p300-reactive protein which migrated at about 160 kDa (Fig. 3A). This smaller anti-p300-reactive protein in RC-K8 cells was expressed approximately ten-fold less than wild-type p300 in A293 cells (Fig. 3A).

Western blotting with the N-terminal p300 antibody showed that p300 is expressed as an apparently full-length protein in the BJAB, BL41, Daudi, IB4, and SUDHL4 human B-lymphoma cell lines (Fig. 3B). As controls, extracts from A293 cells and REL-transformed chicken spleen cells, both of which express full-length p300, were also analyzed. As before, the only p300-reactive protein in RC-K8 cell extracts was the 160-kDa protein, which was less abundant than full-length p300 expressed in other cell lines (Fig. 3B, top panel). The RC-K8-cell 160-kDa protein was not detected by Western blotting with a C-terminal p300 antibody (Fig. 3B, 2nd panel from top). Therefore, this RC-K8-specific protein is likely to be a C-terminally truncated form of p300, herein called p300 Δ C.

The RC-K8 cell line expresses a mutant REL fusion protein (REL-NRG) but no I κ B α protein, due to inactivating mutations [5,28]. To confirm that the cell line used in the current studies was indeed RC-K8, we showed that REL-NRG, but not I κ B α , was expressed in these cells (Fig. 3C).

3.4. p300 Δ C interacted with REL in RC-K8 cells and with REL TAD sequences in vitro

To determine whether p300 Δ C retains the ability to interact with REL, we performed an anti-p300 immunoprecipitation on nuclear extracts from RC-K8 cells. Anti-REL Western blotting showed that REL specifically co-immunoprecipitated with p300 Δ C (Fig. 4A). REL did not, however, interact strongly with CBP in RC-K8 cells, as little REL was detected in anti-CBP immunoprecipitates (Fig. 4B). REL also co-immunoprecipitated with full-length p300 from nuclear extracts of REL-transformed chicken spleen cells (Fig. 4A). These results show that REL interacts with p300 (or p300 Δ C) under conditions where REL has oncogenic activity.

To determine whether p300 Δ C also interacted with REL through the C-terminal TAD, a GST pulldown assay was performed using RC-K8 whole cell extracts. Anti-p300 Western blotting showed that GST-REL-TAD pulled down p300 Δ C, whereas GST-REL-RHD, GST-REL-TAD Δ 1,2, and GST did not (Fig. 4C). These results indicate that, similar to full-length p300, p300 Δ C interacted with REL through the TAD of REL.

3.5. RC-K8 cells expressed an EP300 mRNA truncated at its 3' end

We next performed Northern blotting to determine whether there was an alteration in the size of the normal 9.5-kb p300 mRNA (*EP300*) in RC-K8 cells. A probe complementary to 5'

sequences of *EP300* detected predominantly an *EP300* mRNA from RC-K8 cells that migrated at about 8 kb, whereas wild-type *EP300* mRNA from A293 cells migrated at the expected 9.5 kb (Fig. 5A, left panel). A probe complementary to sequences encoded by exon 31, the last exon of *EP300*, detected the wild-type 9.5 kb *EP300* mRNA from A293 cells, but detected little, if any, *EP300* mRNA in RC-K8 cells after an exposure similar to that of the 5' *EP300* probing (Fig. 5A, right panel). These results indicate that most *EP300* mRNA in RC-K8 cells lacked the wild-type 3' sequences, and strongly suggest that this alteration was responsible for the expression of p300 Δ C.

As a second means of looking at *EP300* mRNA in RC-K8 cells, we performed real-time PCR analysis of cDNA using 5' (exon 2) and 3' (exon 31) primer sets. As compared to A293 cells, most of the *EP300* transcripts expressed in RC-K8 cells lacked exon 31 sequences (Fig. 5B). That is, while *EP300* expression in RC-K8 cells was 77% of that in A293 cells as judged by amplification with exon 2 primers, *EP300* expression in RC-K8 cells was only 16% of that in A293 cells when exon 31 primers were used. This suggests that ~20% (0.16/0.77) of the *EP300* transcripts expressed in RC-K8 were full-length, and that ~80% lacked exon 31 sequences. Similar results were obtained by semi-quantitative RT-PCR comparing amplification of *EP300* cDNA sequences encoded by exons 3–12 to those encoded by exon 31 in A293 vs RC-K8 cells (Fig. 5C).

3.6. Genomic *EP300* sequences were deleted after exon 17 in one allele in RC-K8 cells

To determine whether one of the *EP300* genomic loci in RC-K8 cells contained a deletion, we performed real-time PCR analysis of individual exons (exons 13 through 27) of *EP300* from RC-K8 cell genomic DNA. As controls, we amplified *EP300* exon 2 from RC-K8 cells (which we knew was present in *EP300* mRNA from RC-K8 cells), and we compared the values for each exon amplification from RC-K8 cells to a parallel amplification of *EP300* DNA from BJAB cells (which are not known to contain any *EP300* alteration). The same relative amounts of product were obtained for *EP300* exons 13–17 from BJAB and RC-K8 cell DNA, whereas amplifications from exons 18–27 yielded approximately half the amount of product from RC-K8 cell DNA (Fig. 6A). These results suggest that RC-K8 cells have lost *EP300* sequences after exon 17 on one allele.

4. Discussion

This study reports that the histone acetyltransferases p300 and CBP directly interacted with and acted as transcriptional coactivators for transcription factor REL. Also, p300 and REL were found in a complex in nuclear extracts from both REL-transformed chicken spleen cells and the DLBCL cell line RC-K8, which has constitutively nuclear REL activity. Given that REL's oncogenic activity depends on its ability to activate transcription, these results suggest that the REL-p300 interaction plays a role in REL-dependent transformation. Furthermore, we show that the RC-K8 cell line exclusively expressed a C-terminally truncated version of p300, due to a genomic loss of 3' sequences from one allele of *EP300*. These results represent the first identification of a p300 truncation in a B-lymphoma cell line.

The RC-K8 DLBCL cell line is noteworthy because of the presence of mutations in genes encoding four components of the NF- κ B/REL pathway: REL, I κ B α , A20, and p300. We know of no other example of a tumor cell line that has four mutations which impact a single signaling pathway. First, RC-K8 cells express a C-terminally truncated REL protein, REL-NRG, in which sequences of the RHD are fused to non-REL gene sequences [28,29]; because the TAD sequences are missing in REL-NRG, the protein can no longer activate transcription [5]. Second, RC-K8 cells contain inactivating mutations in the REL inhibitor I κ B α and no I κ B α protein can be detected in these cells; ectopic expression of functional I κ B α inhibits the proliferation of RC-K8 cells [5]. Third, there are inactivating mutations in the gene encoding

A20, an upstream negative regulator of NF- κ B, and expression of wild-type A20 in RC-K8 cells induces apoptosis [30]. Finally, as we show herein, RC-K8 cells express a truncated version of p300, a coactivator for REL-dependent transactivation. Given that chronic, low level REL transactivation is optimal for avian B-cell transformation [7,31], it is likely that RC-K8 cells have fine-tuned their level of REL-dependent target gene transactivation by balancing mutations that positively influence REL activity (mutations in I κ B α and A20) with ones that reduce REL's transactivation ability (REL-NRG and p300 Δ C). In this model, inactivation of I κ B α provides the major impetus for constitutively nuclear REL activity. However, because high level expression of REL is toxic in certain settings [32], mutations that created the DNA-binding competent but transcriptionally inactive REL-NRG protein and the p300 Δ C mutant protein may have been selected to dampen REL-driven transactivation of target genes. In addition, the interaction of p300 Δ C with REL may block the ability of CBP to compensate for the loss of normal p300 co-activating function in RC-K8 cells. No RelA-containing nuclear complexes are detected in RC-K8 cells, suggesting that the constitutive NF- κ B activity is exclusively due to REL complexes [5]. p300 Δ C was also expressed in RC-K8 cells at a much lower level than wild-type p300 in both A293 cells and five B-lymphoma cell lines (Fig. 3A and B).

A20 acts as a negative upstream regulator of NF- κ B activity by deubiquitinating TRAF6 and modifying the ubiquitination of RIP1 [33,34]. Whether A20 mutations contribute to the oncogenic state of RC-K8 cells by promoting unrestrained NF- κ B activity is not clear. That is, expression of wild-type A20 was shown to induce apoptosis in RC-K8 cells [30]; however, it was conspicuously not shown that expression of A20 reduces nuclear NF- κ B complexes in these cells. Indeed, given that there is no I κ B α protein in RC-K8 cells, the reconstitution of normal A20 upstream activity would not be expected to reduce the amount of nuclear REL protein. Thus, at least in RC-K8 cells, A20 mutations may either contribute to oncogenesis by affecting NF- κ B/REL at a downstream step or by affecting other pathways. Indeed, A20, through TRAF6 deubiquitination, also negatively regulates MAPK activity [35].

Based on Northern blotting and quantitative RT-PCR of mRNA and genomic DNA, sequences from exons 18–31 appear to be missing from one of the *EP300* loci in RC-K8 cells. These results indicate that p300 Δ C contains only those amino acid residues encoded by *EP300* exons 1–17 (aa 1-1047); therefore, p300 Δ C would be missing the HAT domain (aa 1224-1669) [36] and the entire C-terminal transactivation domain (Fig. 6A and B). As such, p300 Δ C would be expected to be unable to enhance transcriptional activation by REL because other studies have shown that the HAT domain is required for p300's transcriptional enhancement ability [10,20,36,37]. Also, the N- and C-terminal regions of p300 form a bipartite transactivation domain that recruits TBP, TFIIIB, and RNA polymerase [10], and this function would also be abolished in p300 Δ C. Based on the size of the p300 Δ C protein on SDS-polyacrylamide gels (~160 kDa), we suggest that p300 Δ C is fused to approximately 45 kDa of non-p300 sequences, because exons 1–17 contain 1047 codons, which would yield approximately 115-kDa of p300 polypeptide.

Although our RT-PCR experiments indicate that some wild-type p300 mRNA is expressed in RC-K8 cells (Fig. 5), we have not been able to detect any wild-type p300 protein in these cells. Of note, both wild-type and mutant I κ B α mRNAs are also expressed in RC-K8 cells, but no wild-type I κ B α protein could be detected [5]. These results raise the intriguing possibility that the expression of certain mutant mRNAs (e.g., for p300 and I κ B α) in RC-K8 cells can suppress translation from the corresponding wild-type mRNAs.

To our knowledge, this report provides the first evidence of a p300 truncation in a B-cell lymphoma. Nevertheless, inactivating mutations in p300, many of which result in C-terminal truncations, have been identified in other types of human tumors and tumor cell lines. Previous

reports of p300 truncating mutations have been mostly in cancers of epithelial origin, including colorectal, gastric, breast, pancreatic, cervical, and ovarian [9,10,38,39]. Most cell lines containing p300 truncations have deleted or silenced second *EP300* alleles, and the colorectal cancer cell line HCT116 contains different mutations in its two *EP300* alleles [9,10,39]. Expression of wild-type p300 slows the growth of two cancer cell lines with biallelic inactivating mutations in *EP300* [40]. Moreover, p300 knockout mice develop histiocytic sarcomas [41]. Based on these types of data, p300 has been suggested to be a tumor suppressor. Alterations of p300 have been less frequently found in hematopoietic malignancies. Translocations resulting in fusions of p300 to monocytic leukemia zinc-finger protein (MOZ) in acute myeloid leukemia patients and to mixed lineage leukemia (MLL) in myelodysplastic syndrome patients have been reported [42–44].

Future work is needed to identify the precise genetic alteration that results in expression of the p300 Δ C protein in RC-K8 cells, to determine whether p300 Δ C contains non-p300 sequences, and to determine whether the p300 Δ C protein contributes to the growth, survival, or malignant phenotype of RC-K8 cells. Along these lines, it will be interesting to determine whether overexpression of wild-type p300 or knockdown of p300 Δ C can affect the growth or survival of RC-K8 cells or the interaction of REL with CBP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by NIH grant CA047763 and ARRA supplement CA047763-21S3 (to T.D.G.). G.A. was supported by NSF-REU CHE-0649114. We thank Moritz Brüggemann for help with this work, and Josh Leeman, Dan Starczynowski, and Melanie Herscovitch for helpful discussions.

Abbreviations

aa	amino acid(s)
DLBCL	diffuse large B-cell lymphoma
FLAG	flu antigen
NLS	nuclear localization signal
NRG	non-REL gene
RHD	Rel homology domain
qPCR	quantitative polymerase chain reaction
RT-PCR	reverse transcriptase-PCR
TAD	transactivation domain

References

1. Gilmore TD, Kalaitzidis D, Liang MC, Starczynowski DT. The c-Rel transcription factor and B-cell proliferation: a deal with the devil. *Oncogene* 2004;23:2275–2286. [PubMed: 14755244]
2. Köntgen F, Grumont RJ, Strasser A, Metcalf D, Li R, Tarlinton D, Gerondakis S. Mice lacking the c-*REL* proto-oncogene exhibit defects in lymphocyte proliferation, humoral immunity, and interleukin-2 expression. *Genes Dev* 1995;9:1965–1977. [PubMed: 7649478]
3. Starczynowski DT, Trautmann H, Pott C, Harder L, Arnold N, Africa JA, Leeman JR, Siebert R, Gilmore TD. Mutation of an IKK phosphorylation site within the transactivation domain of REL in

- two patients with B-cell lymphoma enhances REL's *in vitro* transforming activity. *Oncogene* 2007;26:2685–1694. [PubMed: 17072339]
4. Gilmore TD, Cormier C, Jean-Jacques J, Gapuzan ME. Malignant transformation of primary chicken spleen cells by human transcription factor c-Rel. *Oncogene* 2001;20:7098–7103. [PubMed: 11704834]
 5. Kalaitzidis D, Davis RE, Rosenwald A, Staudt LM, Gilmore TD. The human B-cell lymphoma cell line RC-K8 has multiple genetic alterations that dysregulate the Rel/NF- κ B signal transduction pathway. *Oncogene* 2002;21:8759–8768. [PubMed: 12483529]
 6. Martin AG, San-Antonio B, Fresno M. Regulation of nuclear factor B transactivation: implications of phosphatidylinositol 3-kinase and protein kinase C in c-Rel activation by tumor necrosis factor. *J Biol Chem* 2001;276:15840–15849. [PubMed: 11278885]
 7. Starczynowski DT, Reynolds JG, Gilmore TD. Deletion of either C-terminal transactivation subdomain enhances the *in vitro* transforming activity of human transcription factor REL in chicken spleen cells. *Oncogene* 2003;22:6928–6936. [PubMed: 14534540]
 8. Carrasco D, Cheng J, Lewin A, Warr G, Yang H, Rizzo C, Rosas F, Snapper C, Bravo R. Multiple hemopoietic defects and lymphoid hyperplasia in mice lacking the transcriptional activation domain of the c-Rel protein. *J Exp Med* 1998;187:973–984. [PubMed: 9529314]
 9. Iyer ND, Özdag H, Caldas C. p300/CBP and cancer. *Oncogene* 2004;23:4225–4231. [PubMed: 15156177]
 10. Kalkhoven E. CBP and p300: HATs for different occasions. *Biochem Pharmacol* 2004;68:1145–1155. [PubMed: 15313412]
 11. Gerritsen ME, Williams AJ, Neish AS, Moore S, Shi Y, Collins T. CREB-binding protein/p300 are transcriptional coactivators of p65. *Proc Natl Acad Sci USA* 1997;94:2927–2932. [PubMed: 9096323]
 12. Zhong H, Voll RE, Ghosh S. Phosphorylation of NF- κ B p65 by PKA stimulates transcription activity by promoting a novel bivalent interaction with the coactivator CBP/p300. *Mol Cell* 1998;1:661–671. [PubMed: 9660950]
 13. Ravi R, Mookerjee B, van Hensbergen Y, Bedi GC, Giordano A, El-Deiry WS, Fuchs EJ, Bedi A. p53-mediated repression of nuclear factor B RelA via the transcriptional integrator p300. *Cancer Res* 1998;58:4531–4536. [PubMed: 9788595]
 14. Sheppard KA, Rose DW, Haque ZK, Kurokawa R, McInerney E, Westin S, Thanos D, Rosenfeld MG, Glass CK, Collins T. Transcriptional activation by NF- κ B requires multiple coactivators. *Mol Cell Biol* 1999;19:6367–6378. [PubMed: 10454583]
 15. Webster GA, Perkins ND. Transcriptional cross talk between NF- κ B and p53. *Mol Cell Biol* 1999;5:3485–3495. [PubMed: 10207072]
 16. Zhong H, May MJ, Jimi E, Ghosh S. The phosphorylation status of nuclear NF- κ B determines its association with CBP/p300 or HDAC1. *Mol Cell* 2002;9:625–636. [PubMed: 11931769]
 17. Huang WC, Ju TK, Hung MC, Chen CC. Phosphorylation of CBP by IKK promotes cell growth by switching the binding preference of CBP from p53 to NF- κ B. *Mol Cell* 2007;26:75–87. [PubMed: 17434128]
 18. Saha RN, Jana M, Pahan K. MAPK p38 regulates transcriptional activity of NF- κ B in primary human astrocytes via acetylation of p65. *J Immunol* 2007;179:7101–7109. [PubMed: 17982102]
 19. Wang J, Wang X, Hussain S, Zheng Y, Sanjabi S, Ouaz F, Beg AA. Distinct roles of different NF- κ B subunits in regulating inflammatory and T cell stimulatory gene expression in dendritic cells. *J Immunol* 2007;178:6777–6788. [PubMed: 17513725]
 20. Sun H, Lu J, Wei L, Wang X, Xu X, Dong M, Huang B. Histone acetyltransferase activity of p300 enhances the activation of IL-12 p40 promoter. *Mol Immunol* 2004;41:1241–1246. [PubMed: 15482860]
 21. Yu SH, Chiang WC, Shih HM, Wu KJ. Stimulation of c-Rel transcriptional activity by PKA catalytic subunit. *J Mol Med* 2004;82:621–628. [PubMed: 15197457]
 22. Sambrook, J.; Fritsch, EF.; Maniatis, T. *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Press; 1989.
 23. Starczynowski DT, Reynolds JG, Gilmore TD. Mutations of tumor necrosis factor -responsive serine residues within the C-terminal transactivation domain of human transcription factor REL can enhance its *in vitro* transforming ability. *Oncogene* 2005;24:7355–7368. [PubMed: 16027730]

24. Garbati MR, Gilmore TD. Ser484 and Ser494 in REL are the major sites of IKK phosphorylation in vitro: evidence that IKK does not directly enhance GAL4-REL transactivation. *Gene Expr* 2008;14:195–205. [PubMed: 19110719]
25. Schreiber E, Matthias P, Müller MM, Schaffner W. Rapid detection of octamer binding proteins with 'mini-extracts', prepared from a small number of cells. *Nucleic Acids Res* 1989;17:6419. [PubMed: 2771659]
26. Feinberg AP, Vogelstein B. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal Biochem* 1983;132:6–13. [PubMed: 6312838]
27. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 2001;25:402–408. [PubMed: 11846609]
28. Kalaitzidis D, Gilmore TD. Genomic organization and expression of the rearranged *REL* proto-oncogene in the human B-cell lymphoma cell line RC-K8. *Genes Chromosomes Cancer* 2002;34:129–135. [PubMed: 11921291]
29. Lu D, Thompson JD, Gorski GK, Rice NR, Mayer MG, Yunis JJ. Alterations at the *rel* locus in human lymphoma. *Oncogene* 1991;6:1235–1241. [PubMed: 1650444]
30. Compagno M, Lim WK, Grunn A, Nandula SV, Brahmachary M, Shen Q, Bertoni F, Ponzoni M, Scandurra M, Califano A, Bhagat G, Chadburn A, Dalla-Favera R, Pasqualucci L. Mutations of multiple genes cause deregulation of NF- κ B in diffuse large B-cell lymphoma. *Nature* 2009;459:717–721. [PubMed: 19412164]
31. Fan Y, Glinas C. An optimal range of transcription potency is necessary for efficient cell transformation by c-Rel to ensure optimal nuclear localization and gene-specific activation. *Oncogene* 2007;26:4038–4033. [PubMed: 17173064]
32. Bash J, Zong WX, Glinas C. c-Rel arrests proliferation of HeLa cells and affects critical cell regulators of the G1/S-phase transition. *Mol Cell Biol* 1997;17:6526–6536. [PubMed: 9343416]
33. Wertz IE, O'Rourke KM, Zhou H, Eby M, Aravind L, Seshagiri S, Wu P, Wiesmann C, Baker R, Boone DL, Ma A, Koonin EV, Dixit VM. De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF- κ B signaling. *Nature* 2004;430:694–699. [PubMed: 15258597]
34. Lin SC, Chung JY, Lamothe B, Rajashankar K, Lu M, Lo YC, Lam AY, Darnay BG, Wu H. Molecular basis for the unique deubiquitination activity of the NF- κ B inhibitor A20. *J Mol Biol* 2008;376:526–540. [PubMed: 18164316]
35. Walsh MC, Kim GK, Maurizio PL, Molnar EE, Choi Y. TRAF6 autoubiquitination- independent activation of the NF B and MAPK pathways in response to IL-1 and RANKL. *PLoS ONE* 2009;3:e4064. [PubMed: 19112497]
36. Bordoli L, Husser S, Lthi U, Netsch M, Osmani H, Eckner R. Functional analysis of the p300 acetyltransferase domain: the PHD finger of p300 but not of CBP is dispensable for enzymatic activity. *Nucleic Acids Res* 2001;29:4462–4471. [PubMed: 11691934]
37. Liu C, Lu J, Tan J, Li L, Huang B. Human interleukin-5 expression is synergistically regulated by histone acetyltransferase CBP/p300 and transcription factors C/EBP, NF-AT and AP-1. *Cytokine* 2004;27:93–100. [PubMed: 15271374]
38. Gayther SA, Batley SJ, Linger L, Bannister A, Thorpe K, Chin SF, Daigo Y, Russell P, Wilson A, Sowter HM, Delhanty JD, Ponder BA, Kouzarides T, Caldas C. Mutations truncating the *EP300* acetylase in human cancers. *Nat Genet* 2000;24:300–303. [PubMed: 10700188]
39. Bryan EJ, Jokubaitis VJ, Chamberlain NL, Baxter SW, Dawson E, Choong DYH, Campbell IG. Mutation analysis of *EP300* in colon, breast and ovarian carcinomas. *Int J Cancer* 2002;102:137–141. [PubMed: 12385008]
40. Suganuma T, Kawabata M, Ohshima T, Ikeda MA. Growth suppression of human carcinoma cells by reintroduction of the p300 coactivator. *Proc Natl Acad Sci USA* 2008;99:13073–13078. [PubMed: 12237408]
41. Rebel VI, Kung AL, Tanner EA, Yang H, Bronson RT, Livingston DM. Distinct roles for CREB-binding protein and p300 in hematopoietic stem cell self-renewal. *Proc Natl Acad Sci USA* 2002;99:14789–14794. [PubMed: 12397173]
42. Lai JL, Jouet JP, Bateurs F, Deminatti M. Chronic myelogenous leukemia with translocation (8:22): report of a new case. *Cancer Genet Cytogenet* 1985;17:365–366. [PubMed: 3860283]

43. Ida K, Kitabayashi I, Taki T, Taniwaka M, Noro K, Yamamoto M, Ohki M, Hayashi Y. Adenoviral E1A-associated protein p300 is involved in acute myeloid leukemia with t(11;22)(q23;q13). *Blood* 1997;90:4699–4704. [PubMed: 9389684]
44. Kitabayashi I, Aikawa Y, Yokoyama A, Hosoda F, Nagai M, Kakazu N, Abe T, Ohki M. Fusion of MOZ and p300 histone acetyltransferases in acute monocytic leukemia with a t(8;22)(p11;q13) chromosome translocation. *Leukemia* 2001;15:89–94. [PubMed: 11243405]

Fig. 1.

CBP and p300 interact with REL transactivation domain sequences in vitro. (A) Structures of full-length REL (top) and GST-REL fusion proteins used in this study. RHD, Rel homology domain; TAD, transactivation domain; GST, glutathione-S-transferase. (B and C) GST-REL pulldown of endogenous CBP from A293 whole cell extracts. Bound proteins were subjected to anti-CBP Western blotting (top panels). One percent of the amount of extract used for one pulldown (30 μ g) was included on the gel as an input lane. (D) GST-REL pulldown of endogenous p300 from A293 whole cell extracts. Bound proteins were subjected to anti-p300 Western blotting (top panel). For each GST pulldown, Coomassie Blue staining was performed on 5% of GST or GST-fusion protein used in the pulldowns (lower panels).

Fig. 2. p300 and CBP co-immunoprecipitate with REL and act as REL coactivators. (A) Anti-FLAG immunoprecipitation (IP: FLAG) was performed on nuclear extracts of A293 cells that had been transfected with 5 μ g of expression plasmids for the indicated FLAG-REL fusion proteins and 5 μ g of HA-p300. Immunoprecipitates were subjected to anti-HA Western blotting (to detect p300; top panel). For each sample, 1% of the amount of extract used for one IP (5 μ g) was included on the gel as input lane (left 5 lanes). Anti-FLAG Western blotting (WB: FLAG) of 1% of each extract shows approximately equal expression of each FLAG-REL fusion protein (lower panel). * - indicates a nonspecific band. (B) Anti-MYC immunoprecipitation (IP: MYC) was performed on nuclear extracts of A293 cells that had been transfected with 5

µg of the indicated MYC-REL fusions and 5 µg of pCMV-CBP. Immunoprecipitates were subjected to anti-CBP Western blotting (top panel). One percent of the extract used in each immunoprecipitation was included as an input for each sample (left 3 lanes). Anti-MYC Western blotting of 1% of each extract shows approximately equal expression of each MYC-REL fusion protein (lower panel). (C) p300 and CBP enhance REL-mediated transcriptional activation. A293 cells were transfected with 0.5 µg of a 3x-κB-site luciferase reporter plasmid and 0.5 µg of normalization plasmid RSV-βgal. Cells were co-transfected with 0.5 µg of pcDNA-REL or vector alone; and 0.5 µg of HA-p300, or vector alone (left panel). A similar experiment was performed using pCMV-CBP in place of HA-p300 (right panel). Luciferase and β-galactosidase activities were determined, and values were normalized as indicated (1.0). Values are the averages of three experiments performed with triplicate samples.

Fig. 3.

RC-K8 cells express a C-terminally truncated p300 protein. (A) Anti-p300 Western blotting of A293 and RC-K8 whole cell extracts. Twenty-five μ g of A293 extract and 25, 50, 100, and 200 μ g of RC-K8 extract were loaded. (B) The six indicated B-lymphoma cell lines, A293 cells, and REL-transformed chicken spleen cells (REL Spl.) were analyzed by Western blotting for expression of p300 and CBP. Fifty μ g of whole cell extract was loaded, except for RC-K8 extracts, where 50 and 100 μ g were loaded. Western blots were performed with N-terminal (top panel) and C-terminal (2nd panel from top) anti-p300 antisera and with anti-CBP antiserum (3rd panel from top). Coomassie Blue staining shows approximately equal loading of all extracts (bottom panel). (C) RC-K8 cells express a truncated REL protein called REL-

NRG and no I κ B α Forty μ g of A293 and RC-K8 whole cell extract was loaded and anti-REL and anti- I κ B α Western blotting was performed. Anti- β -tubulin Western blotting was performed as a loading control.

Fig. 4.

p300ΔC interacts with REL in RC-K8 cells and with REL TAD sequences in vitro. (A) RC-K8 (top) and REL-transformed chicken spleen (bottom) cell nuclear extracts were immunoprecipitated (IP) with normal rabbit IgG or anti-p300 antiserum. Immunoprecipitates were subjected to anti-REL or anti-p300 Western blotting, as indicated to the side of each panel. One percent (2.5 μg) of the nuclear extract used for immunoprecipitation was included as an input for the anti-REL blots and 10% of the nuclear extract (25 μg) was used as input for the anti-p300 blots. (B) RC-K8 cell nuclear extracts were immunoprecipitated with normal rabbit IgG or anti-CBP antiserum. As in (A), immunoprecipitates were subjected to anti-REL (top panel) or anti-CBP (lower panel) Western blotting with input controls. (C) GST-REL pulldown of p300ΔC from RC-K8 whole cell extract. Bound proteins were subjected to anti-p300 Western blotting (top panel). Coomassie Blue staining was performed on 5% of GST or GST-fusion proteins used in the pulldowns (lower panel).

Fig. 5.

RC-K8 cells express a 3' truncated *EP300* mRNA. (A) Northern blotting using probes from 5' (exons 1–2) and 3' (exon 31) sequences of *EP300* was performed. Fifty, 50, and 20 μ g of A293 RNA and 80, 50, and 20 μ g of RC-K8 RNA were loaded. The positions of the 28S (~4.7 kb) and 18S (~1.9 kb) rRNAs, as determined by methylene blue staining of the filter, are indicated to the side of each Northern blot. (B) Real-time PCR on *EP300* was performed using A293 and RC-K8 cDNA. Specific primer sets for sequences encoded by exons 2 and 31 of *EP300* and for *GAPDH* were then used for PCR amplification. To obtain relative *EP300* mRNA levels, values were normalized to *GAPDH* values (ΔC_t) and then to those of A293 cDNA ($\Delta\Delta C_t$) (1.0). (C) Semi-quantitative RT-PCR on *EP300* was performed using A293 and RC-K8 cDNA. Specific primer sets were used to amplify sequences encoded by exons 3–12 and exon 31 of *EP300*. Band densitometry was measured using ImageJ software. Values were normalized to those of A293 cDNA (1.0). NTC denotes a no template control.

Fig. 6.

EP300 sequences are deleted after exon 17 on one allele in RC-K8 cells. (A) Real-time PCR for individual exons of *EP300* was performed using BJAB and RC-K8 genomic DNA. Specific primer sets for exons 2, 13–25 and 27 were used for PCR amplification of genomic DNA. Values were normalized to those of *EP300* exon 2 (ΔC_t), and then to those of BJAB DNA ($\Delta \Delta C_t$) (1.0). (Exon 26 was omitted because it was difficult to design appropriate primers for this short exon.) (B) Schematic diagram showing the structure of human p300. Residues 1224–1669 constitute the histone acetyltransferase (HAT) domain and the N- and C-terminal regions of p300 form a transactivation domain. The arrow indicates the end of exon 17-encoded residues. It is predicted that p300 Δ C contains only those residues encoded by exons 1–17; as such, p300 Δ C would lack the bromodomain, the HAT domain, and the C-terminal transactivation domain. CH1–3, cysteine/histidine-rich domains; KIX, CREB-binding domain; BD, bromodomain; QP, glutamine/proline-rich.