Regulation of E2A Activities by Histone Acetyltransferases in B Lymphocyte Development*

Curtis Bradney‡, Mark Hjelmeland‡, Yusuhiko Komatsu§, Minoru Yoshida§, Tso-Pang Yao‡, and Yuan Zhuang‡***

From the Departments of Immunology and Pharmacology and Cancer Biology, Duke University Medical Center, Durham, North Carolina 27710, ‡Advanced Life Science Institute, Inc., 1-10-23 Maruyamadai, Wako-shi, Saitama 351-0122, Japan, and §Chemical Genetics Laboratory, RIKEN, Hirosawa 2-1, Wako-shi, Saitama 351-0198, Japan

Genetic studies have demonstrated that the basic helix-loop-helix protein E2A is an essential transcription factor in B lymphocyte lineage commitment and differentiation. However, the mechanism underlying E2A-mediated transcription regulation is not fully understood. Here, we investigated the physical and genetic interactions between E2A and co-activators histone acetyltransferases (HATs) in B cells. Gel filtration analysis of human pre-B cell nuclear extract showed that E2A copurifies with the HATs p300, CBP, and PCAF. A co-immunoprecipitation assay further demonstrated that a fraction of endogenous E2A proteins is associated with each of the three HATs. We show that these HATs acetylate E2A in vitro, enhance E2A-mediated transcription activity, and promote nuclear retention of E2A proteins. A catalytic mutation of p300 completely abrogates the ability of p300 to acetylate E2A and to promote E2A nuclear retention in 293T cells. A breeding test between E2A heterozygous mice and p300 heterozygous mice demonstrated that these two genes interact for proper B cell development. Collectively, these results suggest that E2A and HATs collaboratively regulate B cell development.

The development of B lymphocytes in the bone marrow is initiated and tightly regulated by at least three transcription factors, E2A, EBF, and Pax5 (1). Mice missing any one of these transcription factors show complete block in B cell development at the pro-B cell stage (2–5). Although the expression of EBF and Pax5 are relatively restricted to the B cell lineage, E2A is found to be much broadly expressed. Both biochemical and genetic analyses have indicated that E2A is the most upstream regulator among the three transcription factors and is continuously involved in regulating the expression of B cell-specific genes through the later stages of B cell development (6, 7). It is not clear how E2A controls the broad array of tissue-specific and stage-specific gene expression during B cell development.

E2A is a founding member of the basic helix-loop-helix (bHLH) transcription factor family, which plays an evolutionarily conserved role in regulating the differentiation events in various tissue types including B lymphocytes in mammals (8). The E2A gene encodes two bHLH transcription factors, E12 and E47, which are generated through differential splicing to two adjacent exons that encode the bHLH domains (9). The bHLH domains are required for protein dimerization and DNA binding (10). Two transactivation domains (AD) are mapped to the amino terminus of the E2A proteins (11, 12). E2A proteins form homodimers or heterodimers through HLH interactions with other broadly expressed bHLH transcription factors such as HEB (13) or with tissue-restricted bHLH transcription factors such as MyoD (14). These bHLH protein dimers bind to DNA at the consensus sequence CANNTG, designated as the E-box. Functional E-box sites are found in the promoters and enhancers of a wide variety of tissue-specific genes including immunoglobulin genes in B cells (15).

The ubiquitously expressed histone acetyltransferases (HATs) p300, CBP, and PCAF are transcription co-activators that interact with a broad spectrum of tissue-specific and non-tissue-specific transcription factors (16). Transfection experiments have demonstrated that p300 can associate with the bHLH region (17) and the amino-terminal region (18) of E2A proteins. Studies in yeast further postulate that E2A mediates transcriptional activation through a recruitment of the SAGA (Spt-Ada-Gen5-acetyltransferase)-like chromatin remodeling complex, which contains a HAT (19). However, no study thus far has addressed directly the functional significance of HAT-E2A interaction in B cell development. Here, we provide biochemical and genetic evidence to show that the HATs p300, CBP, and PCAF are regulators of E2A activity in B lymphocytes and the regulation is important for proper B cell development.

EXPERIMENTAL PROCEDURES

Cell Culture and Transfections—The B cell lines REH and NALM6 were maintained in RPMI 1640 supplemented with 10% fetal bovine serum, 100 units/ml penicillin and streptomycin, and 5 mM HEPES. The cell line 293T was maintained in Dulbecco's modified Eagle’s medium supplemented with 10% fetal bovine serum and 100 units/ml penicillin and streptomycin. Cells were transfected using the calcium phosphate transfection method. For transfections, a Myc-tagged E2–5 was generated by adding six repeats of Myc tag to the amino terminus of E2–5. E2–5 is a version of human E2A cDNA that contains the E47 bHLH domain (20). Individual plasmids encoding FLAG-tagged PCAF, Myc-tagged p300, Myc-tagged p300 DY, and FLAG-tagged CBP were generated as described (21).

Gel Filtration—High and low molecular weight standards (Amerham Biosciences) were re suspended in AG buffer (20 mM HEPES, pH 7.0, 100 mM KCl, 0.1 mM EDTA, 20% glycerol) and run through the Superose 6 HR 10/30 column (Amersham Biosciences) using an FPLC...
Acetylation of E2A by Histone Acetyltransferases

85. An amino-terminal truncation was generated by deleting sequence downstream of the 1-14C acetyl coenzyme A (Amersham Biosciences, catalog no. CFA729, 5). The site at the position corresponding to amino acid 401 of E2A was identified by colloidal staining (Invitrogen). Equivalent amounts (1 µg) of each HAT protein were eluted for each experiment. GST-tagged E2–5 and the deletion mutants were cloned into the pGEX vector and were expressed in Escherichia coli. The N-terminal truncation was generated by deleting sequence downstream of the bHLH domain at position corresponding to amino acid 537 of E2–3. Acetyltransferase assays were carried out as described previously (22). Briefly, 1 µg of GST-E2–5 was added to 1 µg of HAT protein purified from transfected 293T cells in 1× HAT buffer with the addition of 1:100 acetyl coenzyme A (Amersham Biosciences, catalog no. CFA729, 5). The reaction was quenched and GST-p53 was used as a control for the presence of E2A. The expression of HAT protein was verified by Western blotting.

In Vitro Acetylation Assay—Each HAT expression plasmid (5 µg) was transfected into 293T cells via the calcium phosphate method. Cells were harvested after 48 h, and HAT proteins were purified from transfected 293T cells using either FLAG (FLAG-CBP, FLAG-PCAF) or Myc (Myc-p300, Myc-p300DY)-conjugated agarose. HAT recovery was verified by colloidal staining and Western blotting of purified protein.

In Vivo Acetylation Assay—DNA (2–5 µg) coding for Myc-E2A and each individual HAT was transfected into 293T cells via the calcium phosphate method. Cells were harvested after 48 h, and Myc-E2A protein was purified from transfected 293T cell extract by Myc-conjugated agarose using the standard immunoprecipitation protocol with the addition of Trichostatin A (5 µM). Immunoprecipitated samples were washed and run on an SDS-PAGE for separation. After protein transfer to a nitrocellulose membrane, the presence of acetylated E2A was detected with the aid of an anti-acetylated lysine antibody. All blots were stripped and reprobed for the presence of E2A. The expression of HAT protein was verified by Western blotting.

In Vivo Acetylation Assay—DNA (2–5 µg) coding for Myc-E2A and each individual HAT was transfected into 293T cells via the calcium phosphate method. Cells were harvested after 48 h, and HAT proteins were purified from transfected 293T cells using either FLAG (FLAG-CBP, FLAG-PCAF) or Myc (Myc-p300, Myc-p300DY)-conjugated agarose. HAT recovery was verified by colloidal staining and Western blotting of purified protein. Equivalent amounts (1 µg) of each HAT protein were used for each experiment. GST-tagged E2–5 and the deletion mutants were cloned into the pGEX-1 vector and were expressed in Escherichia coli. The N-terminal truncation was generated by deleting sequence downstream of the bHLH domain at position corresponding to amino acid 537 of E2–3. Acetyltransferase assays were carried out as described previously (22). Briefly, 1 µg of GST-E2–5 was added to 1 µg of HAT protein purified from transfected 293T cells in 1× HAT buffer with the addition of 1:100 acetyl coenzyme A (Amersham Biosciences, catalog no. CFA729, 5). The reaction was quenched and GST-p53 was used as a control for the presence of E2A. The expression of HAT protein was verified by Western blotting.

In Vivo Acetylation Assay—DNA (2–5 µg) coding for Myc-E2A and each individual HAT was transfected into 293T cells via the calcium phosphate method. Cells were harvested after 48 h, and Myc-E2A protein was purified from transfected 293T cell extract by Myc-conjugated agarose using the standard immunoprecipitation protocol with the addition of Trichostatin A (5 µM). Immunoprecipitated samples were washed and run on an SDS-PAGE for separation. After protein transfer to a nitrocellulose membrane, the presence of acetylated E2A was detected with the aid of an anti-acetylated lysine antibody. All blots were stripped and reprobed for the presence of E2A. The expression of HAT protein was verified by Western blotting.

In Vivo Acetylation Assay—DNA (2–5 µg) coding for Myc-E2A and each individual HAT was transfected into 293T cells via the calcium phosphate method. Cells were harvested after 48 h, and Myc-E2A protein was purified from transfected 293T cell extract by Myc-conjugated agarose using the standard immunoprecipitation protocol with the addition of Trichostatin A (5 µM). Immunoprecipitated samples were washed and run on an SDS-PAGE for separation. After protein transfer to a nitrocellulose membrane, the presence of acetylated E2A was detected with the aid of an anti-acetylated lysine antibody. All blots were stripped and reprobed for the presence of E2A. The expression of HAT protein was verified by Western blotting.

RESULTS

E2A Forms High Molecular Weight Complexes in Transfected HeLa Cells and in Nuclear Extract from the Human Pre-B Cell Line, NALM6—We first used a biochemical approach to evaluate E2A-containing protein complexes in HeLa cells transfected with a Myc-tagged E2–5, a fully functional human E2A cDNA (20). Size exclusion gel filtration of nuclear extract from E2A-transfected HeLa cells eluted E2A-containing complexes in fractions corresponding to molecular sizes greater than the molecular mass of an E2A dimer, −160 kDa. Monomeric E2A protein in whole cell extract was detected by the anti-Myc antibody 9E10 to have a molecular mass of ~80 kDa (Fig. 1A). Western blot analysis indicated that E2A proteins were detected in eluted fractions corresponding to molecular masses as large as 1 MDa and remained present in fractions that eluted down to the monomeric protein molecular weight. These results indicate that E2A is present in complexes consisting of more than just a dimer.

This result prompted us to evaluate further the endogenous E2A proteins present in pre-B cells. Fractions eluted from size exclusion analysis of the human pre-B cell line NALM6 nuclear extract were analyzed by Western blotting for E2A and the histone acetyltransferases p300, CBP, and PCAF. The results indicated that a significant percentage of endogenous E2A is found in a high molecular weight range. The HATs p300, CBP, and PCAF were found co-eluted with E2A in a broad spectrum of molecular mass ranging up to 1 MDa in size (Fig. 1B). On denaturing gels, monomeric E2A, p300, CBP, and a subunit of PCAF were detected with molecular masses of ~70, 300, 300, and 95 kDa, respectively. The detection of E2A and HATs in the same eluted fractions is consistent with previous transfection studies in which E2A was shown to interact with p300 (17, 18).

Endogenous HATs and E2A Interact in Pre-B Cells—Fractionation data suggest that E2A has the potential to interact specifically with each of the three HATs, p300, CBP, and PCAF, in B cells. To investigate this possibility, E2A was immunoprecipitated from the nuclear extract of two pre-B cell lines, NALM6 and REH, using a monoclonal antibody against E2A. After SDS-PAGE analysis of the pull-down products, polyclonal antibodies detected p300, CBP, or PCAF in NALM6 nuclear extract pull-downs (Fig. 2, lane 3) and CBP or PCAF in REH nuclear extract pull-downs (Fig. 2, lane 4). A direct comparison of the band intensities derived from the pull-down products (Fig. 2, lanes 3 and 4) and crude nuclear extracts (Fig. 2, lanes 1 and 2) indicated that approximately one-fifteenth (1/50) of the HAT protein present in the NALM6 nuclear extract was associated with E2A. To confirm the presence of E2A in the pull-down samples, all blots were stripped and reprobed with an anti-E2A antibody to verify the presence of E2A (data not shown). As a negative control, an antibody against the GST protein was not able to pull down each HAT from the two cell lines (Fig. 2, lanes 5 and 6). These results indicate that fractions of E2A are associated with p300, CBP, or PCAF in pre-B cells.

E2A Is Acetylated by p300, CBP, and PCAF in Vitro—Because HATs are known to acetylate lysine residues present in histone and non-histone proteins (16), in vitro acetylation assays were employed to determine whether an enzymatic interaction occurs between E2A and each HAT. Three GST-E2A fusion proteins, the full-length E2–5, a carboxyl-terminal truncation, and an amino-terminal truncation, were used in the acetylation assay (Fig. 3A). GST and GST-p53 were used as a...
negative and a positive control, respectively. The size and equivalence of loading for each GST fusion protein were demonstrated by colloidal blue staining (Fig. 3, lanes 1-3). Western analysis further confirmed that an anti-E2A antibody could recognize all three versions of GST-E2A proteins (Fig. 3B, lanes 5-7). The acetylation assay shows that p300, CBP, and PCAF can acetylate bacterially expressed E2–5 (Fig. 3C, lanes 2, 8, and 14) and the carboxyl-terminal deleted E2–2 (Fig. 3C, lanes 4, 10, and 16) but not the amino-terminal deleted E2–5 (Fig. 3C, lanes 3, 9, and 15). The ability of each HAT to autoacetylate itself demonstrates that the HAT proteins are fully functional and are equally loaded based on band intensities (Fig. 3C, upper panel). Acetylation was also found in several protein degradation products from the E2–2 full-length and carboxy-terminal deletion constructs (Fig. 3C, bands marked with *). It is consistent with the notion that the amino terminus of the E2–5 is acetylated because these protein fragments were recovered from the GST pull-down and thus contain the amino part of the E2–5 proteins. In the same tests, we show that GST-p53 was acetylated (Fig. 3C, lanes 5 and 11) and that GST alone (Fig. 3C, lanes 1, 7, and 13) or E2A without the presence of HAT (Fig. 3C, lanes 6 and 12) was not acetylated. Altogether, this assay demonstrates that the amino half of E2A is a direct target for the acetylation by HATs in vitro.

E2A Is Acetylated by p300, CBP, and PCAF in Vivo—To further investigate HAT-mediated E2A acetylation, we performed an in vivo analysis to determine whether each HAT could acetylate E2A. Antibodies against acetylated lysine were used to determine the acetylation status of E2A in a transfection assay. Myc-tagged E2A proteins were immunoprecipitated with anti-Myc antibodies before being used in the Western analysis. We show that E2A co-transfection with wild-type p300 and CBP in human 293T cells significantly increased E2A acetylation in comparison with E2A alone (Fig. 4A). In addition, the acetylation of E2A by p300 was dependent upon the acetyltransferase activity of p300, as an acetylase-deficient mutation (DY) derived from a human tumor mutation (21) failed to induce E2A acetylation (Fig. 4A, lane 2). Under this assay condition, we did not detect a significant level of acetylation by PCAF even though PCAF was expressed in the transfected cells (Fig. 4B). This experiment did not rule out the possibility that each HAT may interact with E2A in a cell type-specific manner.

Co-transfection of E2A and p300, CBP, or PCAF Increases Transcription Activity—A role for p300 in stimulating the transcription activity of E2A has been shown previously (17, 18). We wanted to evaluate further whether CBP and PCAF could similarly enhance E2A-mediated transcription. Reporter constructs containing four repeats of the E2A binding site (17) were used in two separate transient transfection assays. Consistent with the earlier findings, figure 5 shows that co-transfection of E2A with p300 (lanes 6 and 14) in 3T3 fibroblast cells increases reporter gene expression over E2A alone (lanes 5 and 13). Similar to p300, CBP (lanes 7 and 15) and PCAF (lanes 8 and 16) can also enhance E2A-mediated transactivation of the reporter genes. These results indicated that p300, CBP, and PCAF may play similar roles in modulating E2A activity.

Co-transfection of E2A and p300, CBP, or PCAF Increases the Amount of Nuclear Retained E2A—Studies indicate that acetylation can affect nuclear retention of transcription factors (25). We examined the expression patterns of E2A proteins in 293T cells co-transfected with p300, CBP, or PCAF. Low levels of endogenous E2A expression in 293T cells produced an undetectable background of E2A staining (Fig. 6, A1). In contrast, nuclear and cytoplasmic dispersal of E2A are clearly visible after transfection with E2–5 expression vector (Fig. 6, A2).
Co-expression of p300 (Fig. 6, A3) or CBP (Fig. 6, A5) significantly increased the amount of E2A retained in the nucleus. Co-transfection of PCAF mildly increased nuclear retention of the transiently expressed E2A protein (Fig. 6, A6). Enhanced nuclear retention of E2A was not observed in 293T cells co-transfected with the enzymatic inactive mutant p300DY (Fig. 6, A4). The greatest increase in nuclear retention was observed in cells co-transfected with either E2A and CBP or E2A and p300, which accounted for ~65 and 55%, respectively, of E2A-positive cells (Fig. 6B). Cells co-transfected with E2A and PCAF demonstrated enhanced nuclear retention in ~35% of the E2A-positive cells. In contrast, only 1–2% of the cells showed nuclear retention in transfections with E2A alone or E2A and p300DY, respectively. Expression of individual HAT proteins in transfected cells was verified independently by Western blot assay (Fig. 4B). These results suggest that acetylation of E2A or acetylation of a protein that associates with E2A increases nuclear retention.

**E2A p300 Double Heterozygous Mice Exhibit a Defect in B Cell Development**—It has been shown that mice heterozygous for E2A produce fewer B cells than wild-type controls (2). This phenotype can be enhanced further by mutations in functionally related genes when tested in the compound heterozygous mice (26, 27). A study of p300 heterozygous mice has also revealed a gene dosage effect of p300 on embryonic development (28). To determine whether a functional interaction between E2A and the histone acetyltransferase p300 is required for normal B cell development, we analyzed bone marrow B cells from littermates of a cross between mice that were heterozygous for either E2A or p300. Phenotypic analysis of bone marrow cells of 6–10-day-old littermates demonstrated that E2A and p300 double heterozygous mice had a greater reduction of total B lineage cells (CD19+ B220+) and mature B cells (IgM+B220+) than either of the single heterozygous littermates (Fig. 7, A and B, left panel (Neonates)). This additive effect on bone marrow B cell development seems restricted to E2A and the histone acetyltransferase p300 is required for normal B cell development, we analyzed bone marrow B cells from littermates of a cross between mice that were heterozygous for either E2A or p300. Phenotypic analysis of bone marrow cells of 6–10-day-old littermates demonstrated that E2A and p300 double heterozygous mice had a greater reduction of total B lineage cells (CD19+ B220+) and mature B cells (IgM+B220+) than either of the single heterozygous littersmates (Fig. 7, A and B, left panel (Neonates)). This additive effect on bone marrow B cell development seems restricted to

**Fig. 3. In vitro acetylation of E2A by p300, CBP, and PCAF.** A, diagram of GST E2–5 fusion proteins. The GST fusion proteins contain either the full-length E2–5, E2–5 with the N' truncation, or E2–5 with the C' truncation. The relative positions for the AD2 and bHLH domains are indicated. B, colloidal stain representation of full-length GST-E2–5 (lane 1), C'-deleted GST-E2–5 (lane 2), N'-deleted GST-E2–5 (lane 3), and GST protein (lane 4). Western analysis of GST E2–5 proteins using an anti-E2A antibody detected full-length GST-E2–5 (lane 7), N'-deleted GST-E2–5 (lane 6), and C'-deleted GST-E2–5 (lane 5). The anti-E2A antibody did not detect GST protein (lane 8). FL, full-length E2–5. C, full-length GST-E2–5 was acetylated by p300 (lane 2), CBP (lane 7), and PCAF (lane 12). The C'-deleted GST-E2–5 protein was also acetylated by p300 (lane 4), CBP (lane 9), and PCAF (lane 14). The N'-terminal deleted GST-E2–5 mutant was not acetylated by p300 (lane 3), CBP (lane 8), or PCAF (lane 15). P53 serves as a positive control for acetylation by p300 (lane 5) and CBP (lane 10). GST alone was not acetylated by p300 (lane 1), CBP (lane 6), or PCAF (lane 11). Protein degradation bands are indicated by an asterisk.

**Fig. 4. In vivo acetylation of E2A by p300, CBP, or PCAF.** A, Myc-E2A was immunoprecipitated from transfected 293T cells. Anti-acetylated lysine antibody (upper panel) and anti-E2A antibody (lower panel) were used to detect the acetylated and total E2A proteins present in the immunoprecipitation products loaded on duplicated gels. Samples loaded to the Western gel are E2–5 transfection alone (lane 1) or co-transfection with p300 (lane 2), p300DY mutant (lane 3), CBP (lane 4), or PCAF (lane 5). B, Western blot analysis of HAT expression in transfected cells. Antibodies against p300 (top), CBP (middle), and PCAF (bottom) were used in detecting individual HAT proteins. Small amounts of endogenous p300 (lane 1) and CBP (lane 4) were also detected in the untransfected controls. No endogenous PCAF could be detected in 293T cells (lane 6).
neonates. Analysis of 3–16-month-old adult mice shows a consistent reduction of B cell numbers in E2A heterozygous mice, but the phenotype is not exacerbated in the double heterozygous mice (Fig. 7B, right panel (Adults)). Both neonates and adults show no defect in T cell development (Fig. 7A and data not shown), indicating that the genetic interaction between E2A and p300 is somewhat restricted to the B cell lineage.

DISCUSSION

Previous studies have indicated that the HAT p300 may interact with E2A at multiple, independent sites including AD1 (18, 19), AD2 (18), and the bHLH domain (17). It is generally hypothesized that these interactions allow the enhancer-bound E2A to recruit p300, which subsequently facilitates transcription through acetylation of either histones or the general transcription apparatus (17–19). However, the significance of this interaction in regulating E2A function is still undetermined for normal tissues under physiological conditions. In a series of biochemical studies we have shown that the endogenous E2A proteins interact with p300, CBP, and PCAF acetyltransferases in pre-B cells. We have shown further that E2A is also a target of the enzymatic activity of these HATs. Although the effect of acetylation on E2A function remains to be determined fully, our data suggest that this modification can increase E2A-mediated transcription, which may in part be the result of increased nuclear retention of E2A. Finally, we have provided genetic evidence to show that E2A and p300 interaction is important for normal B lymphocyte development in the bone marrow.

The work presented here is the first to show that E2A is acetylated by three functionally related HATs: p300, CBP, and PCAF. This acetylation of E2A is apparently independent of AD1 because the E2–5 cDNA used in this study lacks the AD1 domain. Using deletion mutants of GST-E2–5, we have determined that the amino half of E2–5 is acetylated by p300, CBP, or PCAF. The amino terminus of E2–5 contains multiple, isolated lysine residues and a cluster of lysine residues at position 80–84 (KKVRK). This lysine cluster along with the nearby sequences is highly conserved between Caenorhabditis elegans and man. However, mutagenesis conversion of these lysine residues to arginine did not prevent E2–5 from being acetylated by HATs (data not shown). Although acetylation of a protein frequently occurs at a site of multiple lysine residues (24, 29, 30), the potential for individual lysine residue involvement cannot be ruled out. Further mutagenesis combinations involving individual lysine residues might aid in determining the site of acetylation.

HAT-mediated acetylation of a transcription factor may alter its DNA binding activity, ability to interact with other proteins, nuclear localization, and protein stability. For example, the acetylation of p53 and GATA1 leads to an increase in sequence-
specific DNA binding and transcription activity (31, 32); acetylation of MyoD leads to a conformational change affecting its ability to recruit coactivators (29); acetylation of HNF-4 leads to increased nuclear localization (25); and acetylation of E2F1 leads to an increase in DNA binding and protein stability (30).

We found that acetylation of E2A did not alter its ability to bind to E box-specific DNA (data not shown). Through transfection experiments we provided two points of evidence that HAT-E2A interaction affects E2A function. First, co-transfection of E2A with each HAT increases E2A-mediated transcription of a reporter gene. Second, transfection experiments have shown that E2A nuclear retention is substantially increased in the presence of HATs. For p300, this increased nuclear retention is dependent on the acetylation activity of p300. This result is consistent with the notion that nuclear retention of E2A may be affected by acetylation. However, our study did not formally rule out the possibility that nuclear retention of E2A is mediated by an unknown protein that is the target of acetylation by
Acetylation of E2A by Histone Acetyltransferases

HAT. In fact, a moderate increase in E2A nuclear retention was observed with PCAF, which does not show significant acetylation activity toward E2A in transfected 293T cells. It is also possible that E2A may be acetylated at several sites by multiple HATs. Each acetylation may affect E2A function in a different way and in a cell type-dependent manner. Although the current work provided important clues, further investigations are required to determine which interaction and modification of E2A is directly relevant to B cell development.

Genetic studies have shown that bone marrow B cells in E2A heterozygous mice are reduced to half the number maintained in wild-type littermates (2). Mice heterozygous for p300 demonstrate a similar gene dosage effect in embryogenesis, differentiation, and cell proliferation (28). Our study has now shown a genetic interaction between these two genes in bone marrow B cell development. A similar type of genetic test has been used previously to confirm functional interaction between E2A and several other transcription factors present in B cells, including HEB, E2–2, and EBF (26, 27). In all cases, a strong correlation between protein interaction and genetic interaction is observed. Although the dosage-dependent phenotype persists from neonates to adult for E2A heterozygous mice, the genetic interaction between E2A and p300 in the compound heterozygous mice was observed only in neonates and not in adults. One explanation for the recovery of B cell numbers in adult compound heterozygotes is the possible compensation for the loss of p300 by other histone acetyltransferases such as CBP, which shares considerable functional and sequence homology with p300 (33). It has been recognized that lymphopoiesis in fetal and neonatal life is regulated differently than in the adult (34). Our data suggest that although E2A is required for B cell development in both young and adult mice, its activity and specificity in regulating B cell-specific gene expression may be modulated by association with different HATs.

The data provided in our study suggest that E2A interact with multiple HATs in B cells. It is conceivable that an individual HAT may be preferentially required for a certain aspect of E2A function at a certain stage of B cell development. It remains a challenge to determine the specificity of these HAT’s in regulating E2A activities in the context of specific E2A target genes during B cell development. The genetic data presented in this report provide not only the first physiological evidence that E2A and p300 collaboratively regulate bone marrow B cell development but also, more importantly, clues for where and when E2A and p300 interaction might have occurred in live animals.

Acknowledgments—We thank Aaron Goldstrohm for advice and assistance on FPLC, Dr. Akhito Ito for reagents in acetylation assay, Dr. Meifang Dai for assistance in mouse breeding and genotyping, and all members of the Zhuang laboratory for comments on the manuscript.

REFERENCES
1. Kee, B. L., Murre, C. (2001) Curr. Opin. Immunol. 13, 180–185
2. Zhuang, Y., Sorian, P., and Weintraub, H. (1994) Cell 76, 875–884
3. Bain, G., Mannig, E., Iston, D., Amsen, D., Kruisbeek, A., Weintraub, B., Krop, I., Schlessin, M., Fenney, A., van Roon, M., van der Valk, M., te Riele, H., Bernes, A., and Murre, C. (1994) Cell 79, 885–892
4. Nott, S. L., Heavey, B., Rolink, A. G., and Bussard, M. (1999) Nature 401, 556–562
5. Lin, H., and Grosschedl, R. (1995) Nature 376, 263–267
6. Quong, M. W., Harris, D. P., Swain, S. L., and Murre, C. (1999) EMBO J. 18, 6307–6318
7. Herblot, S., Aplan, P. D., and Hoang, T. (2002) Mol. Cell. Biol. 22, 886–900
8. Jan, Y. N., and Jan, L. Y. (1993) Cell 75, 827–830
9. Murre, C., McCaw, P. S., and Baltimore, D. (1989) Cell 56, 777–783
10. Voronova, A., and Baltimore, D. (1990) Proc. Natl. Acad. Sci. U. S. A. 87, 4722–4726
11. Quong, M. W., Massari, M. E., Zwart, R., and Murre, C. (1993) Mol. Cell. Biol. 13, 792–800
12. Aronheim, A., Shiran, R., Rosen, A., and Walker, M. D. (1993) Proc. Natl. Acad. Sci. U. S. A. 90, 8063–8067
13. Barndt, R. J., Dai, M., and Zhuang, Y. (2000) Mol. Cell. Biol. 20, 6677–6685
14. Lassar, A. B., Murre, C., Davia, R. L., Voronova, A., Wright, W. E., Baltimore, D., Kadetsch, T., and Weintraub, H. (1991) Cell 66, 305–315
15. Bain, G., Gruenwald, S., and Murre, C. (1993) Mol. Cell. Biol. 13, 3522–3529
16. Goodman, R. H., and Smolik, S. (2000) Genes Dev. 14, 1553–1577
17. Eckner, R., Yao, T. P., Oldread, E., and Livingston, D. M. (1996) Genes Dev. 10, 2478–2490
18. Gu, Y., Sharma, A., and Stein, R. (1998) Mol. Cell. Biol. 18, 2957–2964
19. Massari, M., Grant, P., Pray-Grant, M., Berger, S., Workman, J., and Murre, C. (1999) Molecular Cell 4, 63–73
20. Wilson, R. B., Kiledjian, M., Shen, C. P., Benennra, R., Zullo, P., Dymecki, S. M., Deizler, S. V., and Kadetsch, T. (1991) Mol. Cell. Biol. 11, 6185–6191
21. Ito A., Lai, C. H., Zhao, X., Saito, S., Hamilton, M. H., Appella, E., and Yao, T. P. (2001) EMBO J. 20, 1331–1340
22. Bannister, A., and Kouzarides, T. (1996) Nature 384, 641–643
23. Komatsu, Y., Yukutake, Y., and Yoshida, M. (2002) J. Immunol. Methods, in press
24. Martin, K., Trouche, D., Hagegenie, C., Sorensen, T. S., La Thanque, N. B., and Kouzarides, T. (1995) Nature 373, 691–694
25. Souflouglou, E., Katsikili, N., and Talanidas, I., (2000) Mol. Cell. 5, 745–751
26. Zhuang, Y., Cheng, P., and Weintraub, H. (1996) Mol. Cell. Biol. 16, 2898–2905
27. O’Riordan, M., and Grosschedl, R. (1999) Immunity 11, 21–31
28. Yao, T. P., Oh, S. P., Fuets, M., Zhou, N. D., Ch’ng, L. E., Newsome, D., Bronson, R. T., Li, E., Livingston, D. M., and Eckner, R. (1998) Cell 93, 361–372
29. Starreci, V., Puri, P., Harnamori, Y., Opyrko, Y., Chung, G., Nakatani, Y., Wang, J., and Kedes, L. (1999) Mol. Cell 4, 725–734
30. Martinez-Balbas, M., Bauer, U., Nielsen, S., Brehm, A., and Kouzarides, T. (2000) EMBO J. 19, 662–671
31. Gu, W., and Roeder, R. G. (1997) Cell 90, 595–606
32. Boyes, J., Byfield, P., Nakatani, Y., and Opyrko, V. (1998) Nature 396, 594–599
33. Kung, A. L., Rebel, V. L., Bronson, R. T., Ch’ng, L. E., Sieff, C. A., Livingston, D. M., and Yao, T. P. (2000) Genes Dev. 14, 272–277
34. Rolink, A, Haasner, D, Nishikawa, S., and Melchers, F. (1993) Blood 81, 2290–2300
Regulation of E2A Activities by Histone Acetyltransferases in B Lymphocyte Development
Curtis Bradney, Mark Hjelmeland, Yasuhiko Komatsu, Minoru Yoshida, Tso-Pang Yao and Yuan Zhuang

J. Biol. Chem. 2003, 278:2370-2376.
doi: 10.1074/jbc.M211464200 originally published online November 14, 2002

Access the most updated version of this article at doi: 10.1074/jbc.M211464200

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

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

This article cites 33 references, 16 of which can be accessed free at http://www.jbc.org/content/278/4/2370.full.html#ref-list-1