Human High Mobility Group Box
Transcription Factor 1 Affects Thymocyte Development and Transgene Variegation

Belaid Sekkali, Ewa Szabat, Eleni Ktistaki, Mauro Tolaini, Kathleen Roderick, Nicky Harker, Amisha Patel, Keith Williams, Trisha Norton and Dimitris Kioussis

*J Immunol* 2005; 175:5203-5212; doi: 10.4049/jimmunol.175.8.5203
http://www.jimmunol.org/content/175/8/5203

**References**
This article cites 57 articles, 23 of which you can access for free at:
http://www.jimmunol.org/content/175/8/5203.full#ref-list-1

**Subscription**
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

**Permissions**
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Human High Mobility Group Box Transcription Factor 1 Affects Thymocyte Development and Transgene Variegation

Belaïd Sekkali, Ewa Szabat, Eleni Ktistaki, Mauro Tolaini, Kathleen Roderick, Nicky Harker, Amisha Patel, Keith Williams, Trisha Norton, and Dimitris Kioussis

It has been shown previously that a human CD2 (hCD2) disabled locus control region (LCR) transgene is unable to establish an open chromatin configuration in all the T cells, and this leads to position effect variegation of the transgene. In this study we show that thymus-specific overexpression of human high mobility group box transcription factor 1 (HBP1), a transcription factor that binds a specific sequence within the hCD2 LCR, affects thymus cellularity as well as the number of CD8+ thymocytes in two independent transgenic mouse lines and increases the proportion of T cells that fully activate the transgenic locus in hCD2 variegating mice in a sequence-specific dependent manner. This finding suggests that overexpression of HBP1 can affect lineage commitment and can relieve the suppressive influence of heterochromatin, allowing thymocytes to express the variegating target locus more efficiently. These effects could be the result of direct HBP1 action on LCR activity. Alternatively, the extra HBP1 molecules may sequester repressive elements away from the LCR, thus allowing transcription permissive states to form on the transgene locus. The Journal of Immunology, 2005, 175: 5203–5212.

Transgenic mouse research on gene expression has led to the discovery of a novel class of cis-acting regulatory elements, locus control region (LCR), which acts in cis to ensure that active transcriptional units are established in all cells of a given tissue (1). LCRs can direct the expression of transgenes in a tissue-specific, position-independent, and copy number-dependent manner. Studies of the human CD2 (hCD2) and β-globin LCRs have led to the proposal that LCRs are involved in the establishment and/or maintenance of an open chromatin configuration by overcoming heterochromatin-mediated silencing (1–4).

The hCD2 gene is expressed in all T cells and thymocytes. Studies using transgenic mice have established that a 28.5-kb fragment containing the human CD2 gene and 5 kb of 5′-flanking and 9 kb of 3′-flanking sequences carries all the necessary information for correct tissue-specific expression (5). Within this fragment, a promoter together with a classical enhancer that is located 3′ to the gene have been identified (6). The enhancer coincides with a strong DNase I-hypersensitive site (HSS) called HSS1. Two additional HSSs (HSS2 and HSS3) with no enhancer activity were also identified within the 2-kb 3′-flanking region (2).

Deletion analysis has shown that the 2-kb 3′-flanking region of the hCD2 locus, which contains these clusters of HSSs, is sufficient for hCD2 LCR function (2, 5). Transgenic lines carrying the hCD2 minigene, in which the HSS3 is either partially deleted or omitted, are subject to position effect variegation (PEV) when the transgene is integrated in transcriptionally repressive regions, such as those found in centromeres. PEV is a phenomenon observed with genes that are in close proximity to repressive heterochromatin locations. In such cases heterochromatin is thought to spread to a varying degree in different cells of the same lineage. Consequently, in some of the cells the gene is spared heterochromatinization, and therefore it is expressed, whereas in cells in which the gene has been engulfed by heterochromatin it is silenced. This effect in our PEV model is apparently transgene copy number and orientation independent (2). Furthermore, it appears that the decision on whether to express a hCD2 transgene with a disabled LCR is a stochastic one, and once made, it is maintained through subsequent cell divisions (4). Very similar observations were made in experiments involving other disabled LCRs (3, 7–9).

Disabled LCRs reveal their sensitivity to position effects when integrated into heterochromatic regions of the mouse genome (4, 9). Such chromosomal locations induce a mosaic expression pattern, which bears striking similarity to PEV as described in Drosophila and yeast (10). It has been reported that repeats of transgenes in Drosophila can induce heterochromatinization and subsequently PEV (11). Furthermore, multiple copies of a lacZ transgene reporter under the control of the human β-globin LCR also are subject to PEV. However, the latter result should be seen in the light of numerous studies showing that lacZ gene sequences can have an adverse effect on the transcriptional activity of the transgenic locus (12–15). We have shown previously that multiple copies of a CD2 transgene with a full LCR integrated in the centromere do not variegate, whereas low copy (two to three) integrants of constructs with disabled LCR in the centromere can be severely affected by the surrounding heterochromatin. Conversely, multiple copies of hCD2 with disabled LCR integrated in a favorable chromosomal position do not exhibit variegation (2). Recently, using Cre-loxP technology in our laboratory, we have
shown that reducing the transgene copy number in a variegating mouse increased PEV (A. Williams, manuscript in preparation). Thus, there is strong evidence that variegation of hCD2 transgenes is not related to the number of integrated copies.

Recently, high mobility group (HMG) box transcription factor 1 (HBP1), a member of the HMG box family that is expressed highly in the thymus, was shown by us to bind a region in the hCD2 LCR. Deletion of the HBP1 binding site (FT1) from the LCR leads to PEV when the transgene is integrated in heterochromatin regions (9). We proposed at the time that HBP1 might play a direct or indirect role in chromatin opening and in establishing an active locus. A similar role has been attributed to the erythroid-specific erythroid Kruppel-like factor (16), which is essential for adult β-globin gene expression (17, 18) and is involved, via SWI/SNF family members (16), in the remodeling of the β-globin locus into an active structure (19).

HBP1 is a member of the HMG protein family capable of interacting with specific DNA sequences. The DNA specificity is variable among the members, and based on methylation interference assays performed for lymphoid enhancer factor (LEF)-1T cell factor-1 factor, a consensus heptamer-binding site for sequence-specific HMG boxes (A/T)(A/T)CAAAG was originally proposed (20). Subsequent studies of LEF-1, SRY, SOX-4, and SOX-5 were in agreement with this idea (21–24). However, different binding sites sharing a TCAT tetranucleotide have been reported for HBP1 (25, 26). Among sites that bind HBP1, the TCATTAGTTCA binding site, found in the hCD2-LCR, appears to have the highest affinity (9).

In this study we investigated the effects of increased levels of HBP1 in overcoming heterochromatin-mediated transgene silencing and in thymocyte development by generating transgenic mice overexpressing HBP1 in thymocytes. Such mice exhibit lower thymus cellularity and have increased numbers of mature CD8 single-positive (SP) cells in the thymus. Using two independent hCD2 variegating transgenic reporter lines, it was found that thymus-specific overexpression of HBP1 in two independent HBP1 transgenic effector lines can lead to an increase in the proportion of thymocytes that fully express the transgenic hCD2 locus, provided the HBP1 binding site is present on the reporter construct.

Materials and Methods

Transgenic mouse generation

Mice (CBA/Ca and C57BL/10 and (CBA/Ca × C57BL/10) F2) were bred at the National Institute for Medical Research in London. Transgenic mice were generated by injecting DNA constructs into C57BL/10/CBA at the National Institute for Medical Research in London. Transgenic effector lines can lead to an increase in the proportion of positive (SP) cells in the thymus. Using two independent hCD2 reporter constructs, HBP1 in overcoming heterochromatin-mediated transgene silencing and in thymocyte development by generating transgenic mice overexpressing HBP1 in thymocytes. Such mice exhibit lower thymus cellularity and have increased numbers of mature CD8 single-positive (SP) cells in the thymus. Using two independent hCD2 variegating transgenic reporter lines, it was found that thymus-specific overexpression of HBP1 in two independent HBP1 transgenic effector lines can lead to an increase in the proportion of thymocytes that fully express the transgenic hCD2 locus, provided the HBP1 binding site is present on the reporter construct.

Fluorescence in situ hybridization

Metaphase spreads were obtained from transgenic mouse spleen cells cultured for 2 days after LPS stimulation (Sigma-Aldrich; final concentration, 20 mg/ml). The hCD2 DNA probe was labeled and hybridized with the metaphase spreads following procedures previously described (2). Slides were then mounted in Antifade (Vector Laboratories), counterstained with 4',6-diamidino-2-phenolindole, and subsequently examined using a Delta Vision fluorescence microscope. Images were collected with cooled CCD camera (Photometrics) using capture software (Soft WoRx).

Northern blot analysis

Total thymocyte RNA was extracted using TRIzol reagent (Invitrogen Life Technologies) according to the manufacturer’s instructions. RNA molecules were resolved by agarose gel electrophoresis and transferred onto Hybond-N membranes (Amersham Biosciences) in 20× SSC, 3 M NaCl, and 0.3 M sodium citrate; pH 7.0. Membranes were hybridized to a probe containing the open reading frame of human HBP1 in 3× SSC, 0.1% SDS, 10× Denhardt’s solution, 1% dextran sulfate, and 250 μg/ml denatured salmon sperm DNA at 65°C for 16 h and washed to a final stringency of 0.2× SSC/0.1% SDS at 65°C. As a loading control, the membranes were hybridized to a GAPDH probe as described above.

FACS analysis

T cells from thymus were stained with saturating concentrations of FITC-, PE-, PerCP-, or Tricolor-conjugated Abs in PBS plus 2% FCS-1% BSA at 4°C for 30 min, then washed with PBS and subsequently analyzed by BD Biosciences FACSCalibur and CellQuest programs. The following Abs were used in this study: FITC-anti-mCD8a (YTS169.4), PE-anti-human CD2 (BD Pharmingen), FITC anti-mTCR β-chain (Caltag Laboratories), PerCP-anti-mCD8α (Ly-2; 1/200; BD Pharmingen), allophycocyanin-anti-mCD4 (1/400; BD Pharmingen), and Tricolor-anti-mCD4 (Caltag Laboratories). T cells from the hCD2 nonvariegating line Mg4 and CBA or B10 have been used as positive and negative controls for hCD2 expression, respectively. The experiments shown in Figs. 5 and 6 were performed with a different batch of hCD2 Ab from that used in the rest of the experiments; as a result, the average values from different experiments may appear different for the same line. The comparisons presented in this study are made within the same experiment.

Double-negative (DN) thymocytes were selected by incubating the cells with biotinylated anti-CD8α and anti-CD3ε Abs, followed by streptavidin MicroBeads (Miltenyi Biotec). The cells were stained with PE-conjugated anti-CD8β, anti-TCRβ, anti-Mac1, anti-ter119, anti-CD19, and anti-DX5, and streptavidin (all Abs were from BD Biosciences), and PE-negative cells were sorted according to CD44 and CD25 expression on MoFlo sorter (DakoCytomation).
RT-PCR analysis of RNA

For the HBP1.C line, total RNA from sorted DN subpopulations was prepared with TRIzol (Invitrogen Life Technologies) following the manufacturer’s instructions. For the HBP1.A line, we sorted 50 cells from each DN subpopulation per sample using the MoFlo (DakoCytomation) sorter. In both cases, the RNA was reverse transcribed, and the cDNA was amplified by PCR as previously described (27, 28). Human growth hormone (hGH) primer was used for both the RT reaction and the first PCR. Human GH (nested) was used for the second PCR. HBP1 primer was used in both PCRs. The primers for the hprt gene that was used as an internal control have been described previously (27, 28): HBP1, 5'-GCAAGGCTTGGCTGAGAGAAGC-3'; hGH, 5'-AGGTCTGGCTGAGATCTGC-3'; and hGH (nested), 5'-AAGGCACTGCCCTTGAAGGC-3'.

Results

Generation of HBP1 transgenic mice

We have shown previously in transgenic mice that a deletion of the HBP1-binding sequences in the hCD2-LCR construct leads to PEV when the transgene is integrated in a heterochromatic region (9). We proposed that HBP1 might play a role in chromatin opening and/or remodeling activities by binding to the LCR. To investigate further the effect of HBP1 on chromatin configuration, and consequently on hCD2 LCR function, a cDNA encoding the human HBP1 protein was cloned in the plck expression vector (29) to generate HBP1 transgenic mice (Fig. 1). The plck vector contains the p56lck proximal promoter that is active almost exclusively in thymocytes (30) and has been used to express transgenes specifically in the thymus (29, 31, 32). Three transgenic mouse lines (HBP1A, HBP1B, and HBP1C) with multiple copies of the transgene (n = 45–60) at pericentromeric or telomeric chromosomal sites were generated. We did not detect any rearrangement of the transgenic construct, indicating that most, if not all, integrated copies were intact. We could not detect any expression of the transgene in line HBP1B, and it was therefore discontinued. Expression of the transgene was confirmed by Northern blot analysis of total RNA (Fig. 1) and RT-PCR (not shown). The transgene gives rise
to higher m.w. transcripts compared with the endogenous gene due to the additional exons present in the expression cassette that are spliced onto the HBP1 mRNA.

Effects of overexpression of HBP1 in thymocyte development
Examination of the thymus in HBP1A transgenic mice indicated that the cellularity of this organ was lower than that in age-matched CBA nontransgenic mice. To study the kinetics of this phenomenon, transgenic mice were compared with sex- and age-matched nontransgenic mice at 1 day postpartum and at 1, 3, 6, and 9 wk of age. Fig. 2A shows that newborn transgenic mice had similar cellularity as nontransgenic counterparts. As early as 1 wk later, however, differences became apparent, and these were more noticeable at the ages of 3 and 6 wk. By 9 wk of age, thymus

**FIGURE 3.**  A, Flow cytometric analysis of thymocytes from HBP1.A homozygous and age-matched CBA male mice. Thymocytes were stained with CD4 and CD8 Abs. The percentages of CD4 SP, CD8 SP, and double-positive thymocytes are indicated. Plots show the percentage (B) and absolute numbers (C) of CD8<sup>+</sup>TCR<sup>high</sup> T cells. Each point represents a single mouse. The black lines indicate mean values of the population; numbers in parentheses indicate the number of mice within each group.
cellularity was the same in both groups, possibly because normal involution in wild-type mice had reduced the organ to a similar size. When homozygous transgenic mice were compared with heterozygous littermates, it was found that the former had an even lower cellularity, suggesting that the phenomenon was dose dependent (data not shown). Similar lower cellularity was observed in heterozygous mice of line HBP1C, but the phenomenon was less pronounced (Fig. 2B).

To study the effects of HBP1 overexpression on their development, thymocytes from 6-wk-old HBP1A transgenic mice were isolated and stained for CD4, CD8, and TCR. Results shown in Fig. 3 indicate an increase in the proportion and absolute numbers of CD8 SP thymocytes with high levels of TCR, suggesting that they are postpositive selection CD8 cells. This effect was seen in all groups of mice regardless of sex or age (data not shown). Interestingly, a mild, but opposite, effect was observed for the absolute numbers of CD4 SP thymocytes. Examination of the DP population showed that HBP1 transgenic mice had significantly lower numbers than their nontransgenic counterparts, suggesting that effects on this population account mainly for the lower thymus cellularity seen in these mice (Fig. 3). No increase in CD8 SP T cell number was observed in the periphery. Taken together these results indicate that HBP1 overexpression leads to overproduction of CD8 SP thymocytes despite a decrease in the overall cellularity of the thymus in these mice.

An hCD2 transgene with a disabled LCR exhibits chromosomal PEV

We have shown previously that hCD2 transgenes with only 1.5 kb (CD2-1.5) of the minimum 2-kb LCR sequences (Fig. 4A) exhibit variegating expression (2). This deletion leaves behind a region called FT1, which is recognized by the DNA-binding protein HBP1 (9). We also have shown previously that deletion of a 30-bp sequence including the FT1 region resulted in transgenes that were susceptible to PEV, suggesting that HBP1 might be involved in the protection of the hCD2 transgene from PEV (9).

To investigate directly the involvement of the HBP1 protein in modulating PEV, three new different transgenic mouse lines carrying the CD2-1.5 construct at different positions in their genome were generated (Fig. 4B). Both CD2-1.5A and CD2-1.5E mice

---

**FIGURE 4.** Analysis of CD2-1.5 transgenic mice. A, The hCD2–1.5 construct contains 5 kb of the promoter region, the human CD2 gene with all but the first intron deleted, and 1.5 kb of the 2-kb LCR. The 1.5-kb LCR contains a classical enhancer (HSS1) and the 5′ sequences of a partially deleted HSS3. B, The transgenic hCD2 expression pattern is integration site dependent. Flow cytometric analysis of CD2 expression in CD2-1.5 lines A (35 copies), E (2 copies), and F (20 copies) is represented. Fluorescence in situ hybridization image of metaphase chromosomes, with the transgene highlighted in red (black arrow), shows the integration site in the chromosomal arm (i and ii) or in the centromere (iii).
carry the transgene in the long arm of the chromosome, whereas CD2-1.5F carries the transgene in a pericentromeric region.

The variegating mice did not exhibit discreet expressing and nonexpressing populations; instead, a wide range of expression levels was observed, with only a proportion expressing as high as expected from the number of copies integrated, suggesting that PEV in these mice is not an all-or-none phenomenon involving all integrated copies.

Overexpression of HBP1 increases the proportion of CD2high-expressing T cells in variegating mice

It is possible that PEV reflects incomplete silencing by neighboring heterochromatin, which may be the result, at least in part, of the competition between suppressive and activating chromatin proteins whose competitive efficiency may depend on their relative concentrations (33). In support of this hypothesis, experiments in transgenic mice indicated that overexpression of HP1β (M31) in hCD2 variegating transgenic mice results in an increase in the proportion of T cells that take the decision to silence the transgene (34). Using a similar strategy, the HBP1 factor, which binds to the FT1 region of the LCR, was tested directly for its ability to modulate the transgenic hCD2 locus expression in CD2-1.5A and CD2-1.5F transgenic mouse lines as well as in the nonvariegating CD2-1.5E mouse line. As a control, we used a variegating line (CD2-1.3B) that carried an hCD2 transgene that differs from the constructs present in lines CD2-1.5A, -E, and -F by only a 200-bp deletion containing the HP1 binding site (2). These reporter lines were crossed to the HBP1A and HBP1C lines, and the expression pattern of the hCD2 locus in double-positive thymocytes of double-transgenic mice was compared with that of single hCD2 transgenic mice by flow cytometry (Fig. 5). Single-transgenic CD2-1.5A mice have, on the average, 12% CD2high-expressing thymocytes (Fig. 6A). In double transgenics, an increase to an average of 26.4% was observed in the proportion of CD2high-expressing thymocytes (Fig. 6A). The CD2-1.5F variegating mouse shows an average of 10% CD2high-expressing thymocytes (Fig. 6A). In double transgenics, the increase in HBP1 resulted in an increase in the proportion of CD2high-expressing thymocytes to an average of 21% (Fig. 6A). A similar effect on the extent of variegation was observed with the HBP1C line (Fig. 6B). These results show clearly that HBP1 influences PEV in CD2 variegating mice that carry a CD2-1.5 construct with a disabled LCR. The CD2-1.5E nonvariegating line was also crossed to the HBP1A line, and their analysis showed that overexpression of HBP1 had no effect on the pattern or level of CD2 expression (Fig. 5).

To assess whether the effect of HBP1 overexpression is sequence specific, the hCD2-1.3B variegating line (that does not contain the HBP1 binding site) was crossed to the HBP1A transgenic line. No increase in the proportion of CD2high-expressing thymocytes was found when HBP1 was overexpressed (71.4 vs 72.2%; see Fig. 6C for the average). To confirm that the relief of PEV in CD2-1.5 transgenic mice is dependent on HBP1 binding, we examined the extent of variegation in single-transgenic mice carrying an hCD2 construct with the full LCR minus 30 bp containing the HBP1 binding site (hCD2ΔFT1) and that exhibit variegation (9). We compared these mice with double-transgenic mice (HBP1A/hCD2ΔFT1) and observed no difference in the extent of variegation (Fig. 6D), supporting the idea that the relief of variegation shown in Fig. 5 depends on the presence of an intact HBP1.
FIGURE 6. Flow cytometric analysis of CD2<sup>high</sup>-expressing T cells in the presence or the absence of HBP1 overexpression. A, Plots showing the proportion of CD2<sup>high</sup>-expressing double-positive thymocytes in the single CD2 transgenic and double-transgenic mice overexpressing HBP1 line A. B, Plots showing the proportion of CD2<sup>high</sup>-expressing thymocytes in the single CD2 transgenic and double-transgenic mice overexpressing HBP1 line C. C, Plots showing the proportion of CD2<sup>high</sup>-expressing thymocytes in the single CD2-1.3B and double-transgenic overexpressing HBP1. D, Plots showing the proportion of CD2<sup>high</sup>-expressing thymocytes in the single hCD2-ΔFT1 and double-transgenic overexpressing HBP1. Each point represents a single mouse. The black lines indicate the mean values of the population. n indicates the number of mice, and s.d. represents the SD.
binding site within the LCR. These data demonstrate that HBP1 probably influences PEV of the CD2 locus via the FT1 region localized in the LCR-HSS3. All data of flow cytometric analyses are shown as scatter plots in Fig. 6.

Time variegation of the onset of expression of the lck-HBP1 transgenic constructs

We were puzzled by the observation that the effects of HBP1 overexpression (reduced thymus cellularity, CD8 SP thymocyte overproduction, and relief of hCD2 transgene PEV) were more pronounced in the HBP1-A line despite the fact that it expressed less HBP1 RNA in total thymus preparations. We hypothesized that the explanation may lie in the fact that lck transgenic constructs show variability at the onset of their expression (3, 32). We addressed the issue by sorting DN subsets based on their expression of CD25 and CD44 surface molecules. RT-PCR on RNA isolated from DN1, DN2, DN3, and DN4 populations was analyzed for the expression of transgenic HBP1. As shown in Fig. 7, in the HBP1A line, transcripts appear as early as the DN1 stage in HBP1A mice and only at the DN3 stage in the HBP1.C line. In every case, the hprt gene was used as an internal control of the RT and PCRs.

Discussion

The results presented in this paper show that mice overexpressing HBP1 exhibit altered thymocyte development. The decreased thymus cellularity seen in these mice could be the result of increased apoptosis or decreased proliferation. HBP1 has been shown to affect the cell cycle either positively, by down-regulating cyclin regulator p21 (26), or negatively by blocking the Wnt pathway (35) or by down-regulating the expression of genes such as n-myc (25). We were not able to show any change in apoptosis rates either in vitro or in vivo in these mice (not shown), nor were we able to show any change in the proportion of proliferating cells in transgenic thymuses. Furthermore, analysis of early stages of thymocyte development (DN1 to DN4) does not suggest a developmental block as that seen in other transgenic and knockout mice.

Despite the overall decrease in thymus cellularity, it was surprising to find that deregulation of HBP1 expression led to an increase in the proportion and absolute numbers of CD8 SP mature thymocytes. Increased numbers of CD8 cells have been observed in several other transgenic mice. For example, Bcl2- or TOX-overexpressing transgenic mice show an accumulation of mature CD8 thymocytes for different reasons (36, 37). It is possible that extra HBP1 molecules accelerate the rate of commitment toward the CD8 lineage at the expense of CD4 SP cell production. Interestingly, a phenotype similar to that described in this study has been seen in mice overexpressing a stabilized β-catenin transgene. These mice show enhanced thymic involution and enhanced generation of CD8 SP thymocytes (38). It is therefore possible that overexpression of HBP1, which has been shown to be involved in the Wnt-β-catenin pathway, deregulates this signaling cascade, resulting in faster involution and increased CD8 SP thymocyte production. We hope to be able to address this question in the future.

Cell-to-cell variations in heterochromatin spreading have been proposed to explain the mosaic silencing seen in PEV, with inactivation or full expression indicating whether a heterochromatic structure has encompassed a gene (39). Furthermore, it has been shown previously that variegation in yeast and in mammalian cells can be modified by activating regulatory cis-acting elements, such as promoters or enhancers (40–43). The results of these studies suggest that increased transcriptional activity either helps to prevent the establishment of silencing at the locus or increases the probability that the promoter/enhancer will eventually escape silencing through mitosis (44).

The studies presented in this report indicate that the concentration of HBP1 can influence variegation of the target hCD2 locus. It has been reported that HBP1 can act as transcriptional activator (45, 46) or repressor (25, 26, 35). Although the precise mechanism by which HBP1 deregulation leads to the phenotype we describe is not known, several explanations can be envisaged. One possibility is that HBP1 relieves variegation by helping to enhance expression of the CD2 transgenic locus directly. Such a role has been suggested for other HMG box proteins, such as LEF-1/T cell factor-1, in the regulation of the adenosine deaminase enhancer-LCR (47). It is also possible that HBP1 may be able to disrupt higher order chromatin structure due to its ability to bend DNA via its HMG box domain. DNA bending is a common (but not universal) characteristic of transcription factors, and it is thought to play a role in gene regulation (47–57). However, whether HBP1 acts simply to stabilize interactions at protein-protein interfaces (thereby serving primarily an architectural role), to destabilize the nucleosome array at the integration site, or to directly enhance transcription is unknown.

An alternative explanation for the phenomenon of PEV relief by overexpression of HBP1 could be that normally HBP1 interacts and recruits the LCR-repressive activities that are present in rate-limiting levels in the nucleus and which the disabled LCR cannot counteract. In this scenario, extra molecules of HBP1 might act in a dominant-negative fashion by sequestering the repressor proteins away from the LCR.

It was unexpected that the effects seen in the HBP1.A line are stronger than those seen with line HBP1.C given that the levels of transgenic mRNA are higher in the latter. In this study we show that this may be due to the variable onset of the transgene expression. It has been shown that lck-based vectors exhibit a time variegation (3, 32), and the transgenes in different lines can start expressing at variable time points during thymocyte development.
within the DN stages. We believe that the differences in the magnitude of the effects reflect differences in the time of onset of expression of the lck-HBP1 transgene between lines HBP1.A (onset at DN1) and HBP1.C (onset at DN3).

The results of this study confirm that HBP1 plays an important role in the activation of CD2 transgenic loci by showing that over-expression of HBP1 is related to the proportion of cells that switch on the locus. It will be interesting to assess whether HBP1 mediates this effect at least in part by binding the DNA and/or by interacting with chromatin remodeling complexes with activation or suppression activities.

Acknowledgments
We thank R. Perlmuter for the plck expression vector, Chris Atkins for helping with the sorting, Nicolai Belyev for helpful suggestions, and Henrique Veiga-Fernandes for helping with analysis of the DN populations.

Disclosures
The authors have no financial conflict of interest.

References


