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*J Immunol* 2005; 175:6668-6675; doi: 10.4049/jimmunol.175.10.6668

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*The Journal of Immunology* is published twice each month by The American Association of Immunologists, Inc., 1451 Rockville Pike, Suite 650, Rockville, MD 20852

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Print ISSN: 0022-1767 Online ISSN: 1550-6606.
The Extent of Histone Acetylation Correlates with the Differential Rearrangement Frequency of Individual \( V_H \) Genes in Pro-B Cells

Celia R. Espinoza and Ann J. Feeney

During B lymphocyte development, Ig heavy and L chain genes are assembled by V(D)J recombination. Individual V, D, and J genes rearrange at very different frequencies in vivo, and the natural variation in recombination signal sequence does not account for all of these differences. Because a permissive chromatin structure is necessary for the accessibility of \( V_H \) genes for \( V_H \) to DJH recombination, we hypothesized that gene rearrangement frequency might be influenced by the extent of histone modifications. Indeed, we found in freshly isolated pro-B cells from \( \mu \)MT mice a positive correlation between the level of enrichment of \( V_H S107 \) genes in the acetylated histone fractions as assayed by chromatin immunoprecipitation, and their relative rearrangement frequency in vivo. In the \( V_H 7183 \) family, the very frequently rearranging \( V_H 81X \) gene showed the highest association with acetylated histones, especially in the newborn. Together, our data show that the extent of histone modifications in pro-B cells should be considered as a mechanism by which accessibility and the rearrangement level of individual \( V_H \) genes is regulated. *The Journal of Immunology*, 2005, 175: 6668–6675.

The vast diversity of the immune repertoire is generated during lymphocyte development by assembly of Ag receptor gene segments in a process termed V(D)J recombination. In B cell precursors, there is a precise order of rearrangement of the gene segments, with from the DJH rearrangement occurring before DJH to DJH rearrangement, and H chain rearranging before L chain. In addition, there is lineage specificity in V(D)J recombination. Even though the same recombination-activating gene (RAG) enzymes perform V(D)J recombination in both lineages, only T cells rearrange TCR gene segments, and only B cells rearrange Ig segments.

According to the accessibility model, specific molecular mechanisms should exist in developing lymphocytes that make the appropriate TCR or Ig regions accessible to the common recombinase activity in a lineage- and stage-specific manner. Although it has been demonstrated that cis-acting elements within the TCR and the Ig loci, including enhancers and promoters, play crucial roles in recombination by helping to establish the locus and gene segment-specific accessibility, the precise molecular nature of the DNA or chromatin epigenetic modifications that are responsible for the change in accessibility status of Ig genes is still not fully understood. Chromatin exists in a highly compacted structure in the eukaryotic nucleus, and it has long been accepted that this structure functions as a barrier to gene expression. In general, transcriptionally inactive genes are methylated, show low levels of sensitivity to DNase I digestion, and are associated with hypoacetylated histones, and this is the status of Ig and TCR genes in nonlymphoid cells.

Several studies have demonstrated that demethylation of the H and L chain genes occurs during B cell development, and is associated with onset of V(D)J recombination. Natural variation in the composition of the recombination signal sequence (RSS) is part of the reason for this differential rearrangement, but it does not explain all of the nonrandom rearrangement. For example, our laboratory has shown that the large \( V_H 7183 \) family is composed of many genes that have identical RSS, yet they rearrange at different frequencies in vivo. Thus, it is clear that factors other than RSS efficiency must play an important role in controlling recombination frequency in vivo.

Because induction of histone acetylation has been associated with induction of accessibility to rearrangement for V genes, we postulated that the extent of histone acetylation may be higher for the more frequently rearranging \( V_H \) genes. We therefore examined histone acetylation and methylation by chromatin immunoprecipitation (ChIP), using freshly isolated B220\(^+\) pro-B cells. We found a positive correlation between the extent of the association with acetylated histones and relative rearrangement frequency in the \( V_H S107 \) and \( V_H 7183 \) families. Thus, in the present work, we give the first in vivo demonstration that the extent of acetylation of histone proteins is one of the mechanisms by which V(DJ) rearrangement frequency of individual \( V_H \) genes may be regulated.

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1 This work was supported by a grant from the National Institutes of Health (Grant AI 52313).

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3 Abbreviations used in this paper: RSS, recombination signal sequence; ChIP, chromatin immunoprecipitation; BM, bone marrow; Ct, cycle threshold.
Materials and Methods

Mice

The μMT mice (21) were initially obtained from The Jackson Laboratory, and subsequently bred and maintained in specific pathogenic-free animal facilities at The Scripps Research Institute (La Jolla, CA). Experiments were approved and performed according to the regulatory standards of the Institutional Animal Care and Use Committee.

Cell isolation and B220\(^+\) cell enrichment

Bone marrow (BM) cells were isolated from 6-wk-old μMT mice by flushing the femur and tibia with 10% fetal serum in PBS. Newborn liver cells were obtained from C57BL/6 mice (Charles River, MA). Magnetic cells were stained with anti-B220 conjugated to fluorescent dye (eBioscience), and positive cells were isolated using a magnetic column (Miltenyi Biotec). In both cases, viable pro-B cells (B220\(^+\)) were isolated by flow cytometry. T-lymphocytes were obtained from adult BM and newborn liver of B6 mice. NIH 3T3 fibroblasts were maintained in DMEM (Invitrogen Life Technologies) supplemented with 10% FBS.

PCR, cloning, and sequencing

B220\(^+\) cells from adult BM and newborn liver μMT mice were isolated as described above, and genomic DNA was prepared using QIAamp DNA Mini Kit (Qiagen). Primers used to determine the rearrangement frequency of individual V\(_{\mu}\)S107 and V\(_{\mu}\)J83 genes have been described previously (20, 22). PCR products were ligated into the T/A cloning vector pCR 2.1-TOPO (Invitrogen Life Technologies), and positive clones were sequenced using the ABI PRISM 3100 DNA analyzer (Applied Biosystems).

In vivo ChIP

ChIPs were performed according to Upstate Biotechnology’s protocol, with minor modifications. Approximately 4 x 10\(^6\) cells were crosslinked by adding formaldehyde to a final concentration of 1% to the medium for 15 min at room temperature. The reaction was stopped by adding 0.125 M glycine and incubation for 5 min at room temperature. Subsequent steps were performed at 4°C. Fixed cells were harvested by centrifugation (1,000 rpm for 10 min), washed twice in cold PBS buffer containing 1 mM PMSF and 0.1% protease inhibitor mixture for mammalian extracts (Sigma-Aldrich), and resuspended in cell lysis buffer containing 1 mM PMSF and 0.1% protease inhibitor mixture. After 10-min incubation on ice, lysed cells were centrifuged at 3,000 rpm for 10 min to pellet the nuclei. The nuclear pellet was resuspended in SDS lysis buffer containing 1 mM PMSF and 0.1% protease inhibitor mixture and incubated 10 min on ice. The nuclear suspension was sonicated, and debris was removed by centrifugation (14,000 rpm, 10 min). The chromatin solution was diluted 10-fold in ChIP dilution buffer containing 1 mM PMSF and 0.1% protease inhibitor mixture, and precleared with salmon sperm DNA-protein A agarose beads (Upstate Biotechnology) for 1 h at 4°C. After centrifugation, 500 μl of the supernatant was saved to be used as input DNA, and the rest of the supernatant was incubated overnight at 4°C with 5 μg Ab/10\(^6\) cells. Upstate Biotechnology’s Abs used included anti-acetylated H3 at lysines K9 and K14 (catalog no. 06-599), anti-acetylated H4 at lysines K5, K8, K12, and K16 (catalog no. 06-866), and anti-dimethylated H3 at lysine 4 (catalog no. 07-030).

Immune complexes were collected with salmon sperm DNA-protein A agarose beads for 1 h at 4°C. Following washes and elution, Ab-bound DNA (i.e., DNA associated with the specific histone modification under study) and input DNA (DNA not incubated with any Ab) were reverse crosslinked by heating at 65°C overnight. DNA was recovered after proteinase K treatment, 2 phenol/chloroform/isoamyl alcohol and one chloroform extractions, and isopropanol precipitation. Finally, DNA was dissolved in 1× Tris/EDTA buffer and stored at -20°C until use.

Real-time PCR analysis

Real-time PCR was performed using the Quantitect SYBR PCR Kit (Qiagen) and the ABI Prism 7700 Sequence Detection System (Applied Biosystems). The input DNA and the immunoprecipitated (bound) DNA were first quantified using PicoGreen dye (Invitrogen Life Technologies). The input DNA was diluted in 1× TE to match the concentration of bound DNA. The real-time PCR was conducted using 0.2 ng of DNA at 95°C for 15 min, followed by 45 cycles at 94°C for 15 s, 60°C for 30 s, and 72°C for 30 s. Data were collected at 72°C. A melting curve analysis step was built at the end of the cycling program to verify quality of the PCR products. The sequences of the primers used and their location are described in Table I.

<table>
<thead>
<tr>
<th>Primer Sequence</th>
<th>Position (bp)*</th>
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<tr>
<td>V(_{\mu})S107 family</td>
<td></td>
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<tr>
<td>V1</td>
<td>AGACTGGAGTGATGATTCTGCAAG +130 to +152</td>
</tr>
<tr>
<td></td>
<td>ATGACGCTCTCTCACCTGTGTC +304 to +325</td>
</tr>
<tr>
<td>V11</td>
<td>AGGCCATTAGTTGGTGGGTTT +128 to +150</td>
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<td>V13</td>
<td>TCTGGTCTAGCCTCACAGTGAGTA +316 to +339</td>
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<tr>
<td></td>
<td>CAGCCCTTCCAGGAGGATGCAC +115 to +134</td>
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<tr>
<td></td>
<td>TGTGCTTCTAGCCTACACTGAGTA +318 to +341</td>
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<tr>
<td>V1 regions</td>
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<tr>
<td>5'-Promoter</td>
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<td></td>
<td>GATGTTATTGAGTGGAG +385 to +362</td>
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<tr>
<td>3'-Flanking</td>
<td>CTCTACAACTTTAGTCATGTT +849 to +872</td>
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<td></td>
<td>GATAGAAGCCCTTTAGTGTGTGC +1144 to +1171</td>
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<tr>
<td>Intergenic</td>
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<tr>
<td></td>
<td>AACGATGCTCAGATCTTATTCATG +2923 to +2952</td>
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<tr>
<td>V(_{\mu})J83 family</td>
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<tr>
<td>V81X</td>
<td>AATCCATGATGATGAGATTCTT +68 to +92</td>
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<tr>
<td></td>
<td>CTCGGCGGGGGGGGCTGCTC +351 to +372</td>
</tr>
<tr>
<td>7183 (−81X)</td>
<td>TGGAGCGCCTCTGAGGACTTG +64 to +84</td>
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<tr>
<td></td>
<td>CTCGAGCGGGGGGGGCTGCTC +351 to +372</td>
</tr>
<tr>
<td>V(_{\mu})J558 family</td>
<td></td>
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<tr>
<td>J558</td>
<td>GTGGAGATGTTCCCTGGAAGCTT +52 to +75</td>
</tr>
<tr>
<td></td>
<td>GAGATTGTGCTGAGTGGGAGTTC +198 to +225</td>
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<td>Controls</td>
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<td>Actin</td>
<td>AGCGATGAGGGCTGCTGCTGCTGATC +527 to +548</td>
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<tr>
<td></td>
<td>AGCGAGTGATGGTGGAGGTGAGG +736 to +756</td>
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<td>β2-microglobulin</td>
<td>CTCGAGGCGGCTGCTGCTGAGC +305 to +283</td>
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<td>CCGGCTGCTGCTGCTGAGCTG +158 to +131</td>
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<td>Neuregulin</td>
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<tr>
<td></td>
<td>AGAGGGAGGGCTTGCTAGACAG +248 to +271</td>
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* Numbering relative to the start of the coding region (+1).

† Primers for β2-microglobulin were taken from Chowdhury and Sen (16).
Table I. The specificity of each PCR was first confirmed by sequencing the amplified product.

Enrichment of active genes in our ChIP preparations was assessed by real-time PCR using primers for active genes (actin and β2-microglobulin), and for an inactive gene (neuregulin).

Data is presented as relative to actin values for each amplified DNA sequence, which was determined as follows: 2^((Ct input sample - Ct bound sample) - (Ct input actin - Ct bound actin)), where Ct is the cycle threshold.

Data analysis

Real-time PCRs were performed two to three times per ChIP preparation. All data used in figures are expressed as the mean values of three to six independent ChIP preparations ± SEM. The Mann-Whitney U test was used for statistical evaluation of the results.

Results

The VγS107 genes rearrange at different frequencies

The μMT mice (21) have a targeted disruption of a membrane exon of the gene encoding the IgM constant region, resulting in a complete block of B cell development at the pro-B cell step. Therefore, this strain of mice is an ideal model to analyze the initial rearrangement frequency of VH genes.

The VγS107 family has three functional members, V1, V11, and V13. We previously described that in newborn liver B220+ pro-B cells from μMT mice, V1 rearranges five times more often than V11, and V11 rearranges 7.7-fold more often than V13 (22). In this study, we performed a similar analysis in adult BM B220+ pro-B cells from μMT mice to determine whether the rearrangement frequency of individual members changes from newborn to adult B220+ cells. We isolated newborn liver and adult BM B220+ pro-B cells from μMT mice and amplified the VγS107 genes as described previously (22). The sequencing of additional 15 clones from newborn B220+ pro-B cells did not change the differential rearrangement frequency previously observed within the members of the Vγ S107 family (Fig. 1). In addition, sequencing of 45 clones from adult BM B220+ pro-B cells showed approximately the same rearrangement frequency pattern as observed in newborn liver pro-B cells (V1>V11>V13) (Fig. 1). Thus, this unequal rearrangement pattern is stable throughout ontogeny.

Modification of histones associated with the VγS107 genes

Next, we wanted to test our hypothesis that the extent of histone modifications may correlate with rearrangement frequency in vivo. ChIP assays using Abs to acetylated histone H3, acetylated histone H4, or histone H3 dimethylated at lysine 4 (H3/K4) were performed on freshly isolated B220+ pro-B cells from adult BM and newborn liver from μMT mice. Primers unique for individual members of the VγS107 family were used in real-time PCR. The forward primers were located in the coding region, and the reverse primers were situated 3′ of their RSS. Thus, only unrearranged genes were assayed.

In adult BM B220+ pro-B cells, we found a positive correlation between the rearrangement frequency of the VγS107 family members, and their level of enrichment in the DNA immunoprecipitated with Abs against acetylated histones H3 and H4, and dimethylated H3/K4 (Fig. 2, A–C). V1 has 3- and 2.4-fold higher levels of histone H3 and H4 acetylation, respectively, compared with V11 (p < 0.10 and p < 0.05, respectively; Fig. 2, A and B). Furthermore, V11 has 2.5- and 2.9-fold higher levels of histone H3 and H4 acetylation, respectively, compared with V13 (p < 0.05 and p < 0.02, respectively). For dimethylated H3/K4, V1 was 6.2-fold more enriched in the immunoprecipitated fraction than V11, and V11 was 2.7-fold more enriched than V13 (p < 0.10; Fig. 2C).

In newborn B220+ cells, the rearrangement frequency also correlated with association with acetylated histone H3 (Fig. 2D). V1 has 1.8- and 2.2-fold higher levels of histone H3 acetylation associated with its RSS region than that of V11 and V13, respectively. Although we could not find a significant difference between V1 and V11 in our anti-acetylated H4-ChIP preparations (Fig. 2E), V11 was ~2-fold more enriched in the immunoprecipitated fraction than V13 (p < 0.10).

We conclude that in adult pro-B cells, there is a correlation between the extent of histone H3 and H4 acetylation and H3/K4 dimethylation, and relative rearrangement frequency for the three Vγ genes in the VγS107 family, and this is also the case for acetylated H3 in newborn pro-B cells.

![FIGURE 1. In vivo relative rearrangement frequency of the three VγS107 genes. PCRs with family-specific primers were done on DNA from newborn liver and adult BM B220+ pro-B cells from μMT mice, and the PCR products were cloned and sequenced. Results are expressed as the percentage of the total number of the VγS107 family rearrangements.](http://www.jimmunol.org/)

![FIGURE 2. Analysis of histone acetylation and methylation in the VγS107 family. ChIP assays were performed on adult BM pro-B cells from μMT mice using Abs to acetylated H3 (A), acetylated H4 (B), or histone H3 dimethylated at lysine 4 (H3/K4) (C). ChIP assays were also performed on newborn liver pro-B cells from μMT mice using Abs to either acetylated H3 (D) or acetylated H4 (E). Analysis was performed by real-time PCR. Data is expressed relative to the positive control actin gene (actin = 1). Results represent the mean ± SEM of 3–5 independent ChIP preparations.](http://www.jimmunol.org/)
**Histone modification is localized to the rearranging VH gene**

We wanted to assess whether histone proteins H3 and H4 associated with VH-flanking regions were acetylated or dimethylated to the same degree as the histones surrounding the RSS and coding regions. For this analysis, three different regions (5′-promoter, 142–421 bp upstream of leader; 3′-flanking region, 504–822 bp downstream of the RSS; and intergenic region, 2.4–2.6 kb downstream of the RSS) flanking the VH gene were analyzed in adult BM B220⁺ pro-B cells. Histone H3 and H4 acetylation, as well as H3/K4 dimethylation reached their highest value around the coding region and RSS, decreasing dramatically within 1 kb 5′ and 550 bp 3′ of the RSS (Fig. 3, A–C). This data shows that the extent of histone modifications associated with accessible chromatin is localized to the rearranging gene.

**Higher rearrangement of VH81X gene in newborn than in adult B220⁺ cells**

It has been well documented that VH81X, a member of VH7183 family, is the most frequently rearranging VH segment in fetal life (23–26). We have previously shown that in newborn B220⁺ pro-B cells, VH81X clearly dominates the VH7183 repertoire, comprising 59% of the rearrangements (20). In this study, we performed a similar analysis in B220⁺ adult BM cells to determine the rearrangement frequency of VH81X with respect to the rest of the VH7183 family members.

Rearranged VH7183 genes from adult BM B220⁺ pro-B-cells from μMT mice were amplified, cloned, and sequenced. Of 31 clones derived from adult BM B220⁺ pro-B cells, VH81X accounted for 35% of the rearrangements in the VH7183 family (data not shown). The remainder of the VH7183 genes showed similar relative rearrangement frequency as in the newborn (data not shown). Although these data indicated that VH81X rearranges more often than any other VH7183 gene in both newborn and adult B220⁺ cells, the relative rearrangement frequency of the VH81X gene was much higher in neonatal than in adult B220⁺ pro-B cells.

**Higher association of acetylated histones with VH81X**

To test our hypothesis that the level of enrichment of acetylated histones surrounding VH81X will be higher than that of the rest of the VH7183 family members, ChIP assays were performed and analyzed by real-time PCR using primers that specifically amplified the VH81X gene and primers that amplified the rest of the VH7183 family members, excluding the VH81X gene. The forward primers were located in the coding region, and the common reverse primer was located 37 bp downstream of the nonamer.

In adult pro-B cells from μMT mice, VH81X has 2.4- and 2-fold higher level of histone H3 and H4 acetylation, respectively, compared with the rest of family members (p < 0.02 and p < 0.05, respectively; Fig. 4, A and B). Interestingly, in newborn pro-B cells from μMT mice, there was even more preferential association of the VH81X gene with acetylated histone H3, 4.2-fold higher than that of the rest of family members (p < 0.10; Fig. 4C). However, we could not detect a significant difference between VH81X and the rest of VH7183 family members in the acetylated H4-immunoprecipitated fraction in newborn liver pro-B cells (Fig. 4D). Thus, VH81X has a higher level of histone H3 acetylation associated with its RSS region than that of the rest of the VH7183 genes, and this differential association is much greater in the newborn, correlating with its very high rearrangement frequency.

**Acetylation and methylation status of histones associated with the VHJ558 family**

The large VHJ558 family, which is the most distal family in the VH locus, was included in our global analysis of the histone acetylation and methylation status in adult and newborn B220⁺ pro-B cells. VHJ558 genes undergo rearrangement much less frequently

**FIGURE 3.** Acetylation and methylation patterns of histones H3 and H4 associated with flanking regions of the VH gene in pro-B cells from adult μMT mice. ChIP assays were performed using Abs to acetylated H3 (A), acetylated H4 (B), or dimethylated H3/K4 (C). Real-time PCR was performed using primers designed to amplify four different VH regions (5′-promoter, coding and RSS, 3′-flanking and intergenic). Data is expressed relative to the positive control actin gene (actin = 1). Results represent the mean ± SEM of five independent ChIP preparations.

**FIGURE 4.** Analysis of histone acetylation in the VH7183 family. ChIP assays were performed on adult BM pro-B cells from μMT mice using Abs to either acetylated H3 (A), or acetylated H4 (B). ChIP assays were also performed on newborn liver pro-B cells from μMT mice using Abs to either acetylated H3 (C) or acetylated H4 (D). Analysis was performed by real-time PCR. Data is expressed relative to the positive control actin gene (actin = 1). Results represent the mean ± SEM of 4–6 independent ChIP preparations.
in fetal and neonatal pro-B cells than in adults (27). Because the status of histone proteins associated with the \( V_{H}J558 \) family has not been analyzed in freshly isolated neonatal pro-B cells, we examined this \( V_{H} \) family by ChIP assay using newborn liver and also adult BM pro-B cells from \( \mu MT \) mice. For our real-time-PCR analysis, we designed a pair of primers that would amplify most of the \( V_{H}J558 \) family members, with primers located in FR1 and FR3.

The distal \( V_{H}J558 \) family showed higher association with acetylated histone H3 in adult than in newborn pro-B cells (\( p < 0.10; \) Fig. 5A), which correlates with the high rearrangement frequency of \( V_{H}J558 \) genes in adult pro-B cells. However, when comparing the extent of H3 and H4 acetylation surrounding different \( V_{H} \) genes in newborn liver pro-B cells, the \( V_{H}J558 \) family was more associated with acetylated H3 than any of the proximal \( V_{H} \) genes other than \( V_{H}81X \), which showed the highest association with acetylated histone H3 (Fig. 5A). Furthermore, there was a higher association of acetylated H4 with distal than with proximal \( V_{H} \) genes in both adult BM and newborn liver pro-B cells (Fig. 5B). In addition, in adult pro-B cells, the \( V_{H}J558 \) family had a much higher association with dimethylated H3/K4 than the other \( V_{H} \) genes analyzed (Fig. 5C).

We conclude that the extent of histone H3 acetylation among \( V_{H} \) genes is more comparable in newborn than in adult pro-B cells. Unlike in the newborn, in the adult the most notable difference is the very high level of H3 acetylation and H3/K4 dimethylation of the distal \( V_{H}J558 \) family, and, to a much lesser extent, \( V1 \). Thus, in general, the extent of histone modifications correlates with the relative rearrangement frequency in vivo.

**Increased level of histone acetylation is specific for pro-B cells**

To demonstrate the lineage specificity of the acetylation of histones associated with \( V_{H} \) genes, we performed ChIP on freshly isolated \( \mu MT \) thymocytes and on cultured NIH 3T3 fibroblasts. We found that 3T3 cells displayed little acetylation of histones associated with any of the \( V_{H} \) genes analyzed (Fig. 6, A and B). Furthermore, although thymocytes showed a slightly increased level of H3 acetylation as compared with NIH 3T3 cells, they were still hypoacetylated compared with pro-B cells. Thus, induction of hyperacetylation is specific to the B lineage. In addition, this comparative study demonstrated that it is only in pro-B cells where the level of association of individual \( V_{H} \) members with acetylated histones correlates with their rearrangement frequency (Fig. 6, A and B).

**Discussion**

To elucidate the epigenetic mechanisms regulating accessibility of individual \( V_{H} \) genes to V(D)J recombination, we investigated the modification status of histone proteins associated with specific \( V_{H} \) genes in pro-B cells. We had already shown that the three functional members of the \( V_{H}S107 \) family have very different rearrangement frequencies in vivo, with \( V1 \) rearranging five times...
more than V11, and V13 rearranging 40 times less frequently than V1 (22). Although their RSS share the same heptamer and nonamer (28), we have previously determined that the difference in recombination frequency between V1 and V11 could partially be due to differences in the spacer sequence (29). However, other factors in addition to RSS efficiency must affect recombination frequency because the closely related V11 and V13 genes rearrange at very different frequencies, and their RSS are identical, including the spacer (22, 29). In addition, we previously showed that the promoters of V11 and V13 are similar in strength, as measured by luciferase assays in the presence and absence of the intronic H chain enhancer (22). Furthermore, we showed that the steady-state level of germline transcripts was infrequent for all three V\textsubscript{\mu}S107 genes in vivo, precluding precise quantitation of the relative frequency of transcripts from the individual genes (22).

Another V\textsubscript{\mu} gene family in which RSS differences do not account for rearrangement frequency is the large V\textsubscript{\mu}7183 gene family. Although sequencing of the RSS of all 20 V\textsubscript{\mu}7183 family members indicated that several have identical RSS (20), genes with identical RSS rearranged at very different frequencies in vivo. The V\textsubscript{\mu}7183 gene family contains V\textsubscript{\mu}81X, a gene that rearranges at an extraordinarily high frequency, especially in fetal life. V\textsubscript{\mu}81X is the most 3' functional V\textsubscript{\mu} gene, and shares the same consensus heptamer with other family members, although it has a unique spacer sequence. However, we previously demonstrated that the very high rearrangement frequency of V\textsubscript{\mu}81X is not due to its RSS (20). Therefore, it is clear that factors other than RSS efficiency must play a role in controlling V\textsubscript{\mu} gene rearrangement frequencies.

Increasing evidence that acetylation of histone proteins H3 and H4 and dimethylation of histone H3 at lysine 4 are associated with active chromatin led us to hypothesize that genes that rearrange more frequently within a V\textsubscript{\mu} family may be more enriched in histones that have active posttranslational modifications. Consistent with this idea, we show in freshly isolated pro-B cells a positive correlation between rearrangement frequency among the V\textsubscript{\mu}S107 and V\textsubscript{\mu}7183 family members and their level of enrichment in acetylated histone fractions. For the V\textsubscript{\mu}S107 family, we found that in adult BM pro-B cells the extent of association with acetylated histones H3 and H4, as well as dimethylated H3/K4, was higher with the highly rearranging V1 gene than with V11 gene. This result indicates that in addition to the contribution of the RSS spacer sequence in the higher rearrangement frequency of V1 over V11, association with acetylated H3 and H4, as well as dimethylated H3/K4, may also play a role in the unequal rearrangement frequency of these two V\textsubscript{\mu}S107 genes. Our data also suggest that the difference in recombination frequency between V11 and V13 in adult pro-B cells could be influenced by a preferential association of V11 with acetylated H3 and H4, and dimethylated H3/K4 histones. Our data in this study uncover the first difference between V11 and V13 genes in adult pro-B cells.

In the newborn, however, the extent of histone H3 and H4 acetylation was not significantly different between V1 and V11, and the high rearrangement frequency of V11 over V13 correlated only with the association of V11 with acetylated histone H4. Thus, additional factors may be responsible for the differential rearrangement frequency within V\textsubscript{\mu}S107 family members in newborn pro-B cells.

In the present work, we also showed that the high rearrangement frequency of V\textsubscript{\mu}81X with respect to the rest of V\textsubscript{\mu}7183 family members could be influenced by its very high association with acetylated histone H3. That association was even more pronounced in newborn than in adult pro-B cells, which concurs with the extremely high rearrangement frequency of V\textsubscript{\mu}81X early in ontogeny. However, we did not observe much dimethylation of H3/K4.
provided evidence for at least two roles of the transcription factor PaPa5: facilitating rearrangement of distal V_{H} genes by inducing IgH locus contraction (45), and also in removing H3 methylation at lysine 9, a marker of repressed chromatin, in the V_{H} locus, making V_{H} genes accessible for V_{H} to DJ_{H} recombination (46). Thus, it is likely that accessibility to V(D)J recombination during early B cell development is controlled by several factors.

There are at least two explanations for the observations presented in this study. The accessibility of individual V_{H} genes could be determined by the relative number of acetylated lysines on histones associated with individual V_{H} genes, and/or by the proportion of pre-B cells carrying a particular V_{H} gene in an active chromatin environment. The later proposal has been reinforced by the observation that the activation of germline k transcription occurs only in a small fraction of otherwise apparently homogeneous pre-B cells (47). Furthermore, preliminary ChIP data in our laboratory indicate that only a small fraction of V_{K} genes are acetylated in BOSC cells after transfection with E2A, yet the frequency of induced recombination of those genes was high, suggesting that the V_{K} genes associated with acetylated histones are the ones accesible for rearrangement (P. Goebel, M. Cherrier, and A. J. Feeney, unpublished data). Thus, we propose that it is most likely that an increased proportion of pre-B cells have acetylated histone proteins surrounding frequently (e.g., V_{L} and 8/I_{X}) vs infrequently rearranging genes.

Taken together, our data show that the extent of acetylation of histone proteins is one of the mechanisms by which V(D)J rearrangement frequency of individual V_{H} genes may be regulated in B cell precursor B cells. CHO genetics and tissue-specific expression of unrearranged VH gene segments. Cell 60: 271–281.


