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S L Giannini, M Singh, C F Calvo, G Ding and B K Birshtein

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DNA Regions Flanking the Mouse Ig 3'α Enhancer Are Differentially Methylated and DNAse I Hypersensitive during B Cell Differentiation

Sandra L. Giannini, Mallika Singh, Charles-Félix Calvo, Guifeng Ding, and Barbara K. Birshtein

Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY 10461

ABSTRACT. Two B cell-specific enhancer elements are associated with the IgH gene cluster. One enhancer is located within the J-Cp intron (Ep), whereas a second enhancer (3'αE) is approximately 12.5 kb 3' of the Ca membrane exon. In an attempt to understand the function of 3'αE, we have characterized its surrounding structural milieu during various stages of B cell differentiation through analysis of methylation patterns and the identification of DNAse I-hypersensitive sites. We observed a correlation between the chromatin structure of this region and the differentiation state of the cell. Compared to liver and brain, the region 3' of α was hypermethylated in pre-B and T cell lines and became progressively demethylated as B cell differentiation continued. A DNAse I-hypersensitive site was present in pre-B cell lines about 17 kb 3' of 3'αE. In fully differentiated myeloma cell lines, a second cluster of DNAse I-hypersensitive sites was present immediately 5' of 3'αE. Our data indicate that the 3'α enhancer is relatively sequestered during early stages of B cell differentiation and becomes increasingly accessible at later stages. Journal of Immunology, 1993, 150: 1772.

The mouse H chain enhancer, Ep, has been shown to be important for the tissue-specific expression of IgH genes during B cell development (1). However, we and others have shown that IgH genes could be efficiently transcribed in the absence of Ep (2-5). These observations indicated that either another regulatory element within the IgH gene cluster could substitute for the deleted enhancer or that once IgH expression was initiated, expression could be maintained independent of any enhancer element. Experiments showed that an enhancer—in this case, Ep—was necessary for both the initiation and maintenance of IgH gene expression in pre-B cells (6, 7). By inference, it seemed likely that at all stages of B cell differentiation (pre-B, B, and plasma cells), an enhancer was required for the continued expression of IgH gene expression. Together, these results supported the assumption that an additional B cell-specific regulatory element was contributing to H chain expression in the absence of Ep.

Recently, a second B cell-specific enhancer element was located 3' of the IgH cluster in both rat (26 kb 3' of Ca) (8) and mouse (12.5 kb 3' of Ca membrane exon) (9, 10).
In contrast to Eμ, which is located in the J-ε intron within a few kilobases of the expressed Vμ gene, the 3'-enhancer is located at least 150 kb 3' of the Vμ promoter in rat and mouse.

To understand the potential role of the 3'α enhancer (3'αE) in IgH gene expression during B cell development and its relationship to the function of Eμ, we have examined structural properties of the region flanking 3'αE by analyzing methylation patterns and DNase I-hypersensitive sites. Methylation of DNA has been demonstrated to interfere with gene expression (11-13). DNase I hypersensitivity detects alterations in chromatin conformation associated with promoters, enhancers (14, 15), locus-activating regions (16), and matrix attachment sites (17). These alterations are thought to represent sites of DNA protein interactions that regulate gene expression (18).

Our results reveal B cell stage-specific patterns of methylation and DNase I-hypersensitive sites within the region 3' of Ca. These data imply that the 3' enhancer is relatively sequestered during early stages of B cell differentiation, whereas at later stages it becomes increasingly accessible.

Materials and Methods

Cell lines

Cell lines used were 18-81 (BALB/c; pre-B), 3-1 (BALB/c; pre-B), W231 (BALB/c × NZB; B cell lymphoma; μ,κ), BCL-1 (BALB/c; B cell leukemia; μ,δ,λ), TEPC 1017 (BALB/c; myeloma; δ,κ), NSO (BALB/c; myeloma), MPC11 (BALB/c; myeloma; γ2b, κ), F5.5 (BALB/c; myeloma; γ2b,κ), J558 (BALB/c; myeloma; α,λ), S107 (BALB/c; myeloma; α,κ), W3129 (BALB/c; myeloma), α,κ), BW5147 (AKR/J; T cell lymphoma), and EL-4 (C57BL/6N; T cell lymphoma). Myeloma cells and B hybridomas were maintained in Dulbecco's modified Eagle's medium (GIBCO) with 20% heat-inactivated horse serum. Pre-B and B cell lines were maintained in RPMI 1640 (Whitaker) with 10% heat-inactivated FCS (GIBCO) containing 50 μM β-mercaptoethanol (β-ME). The T lymphoma lines were maintained in Dulbecco's modified Eagle's medium with 10% FCS. In addition, all media contained 100 U/ml of penicillin-streptomycin and 2 mM L-glutamine. Cells were maintained at 37°C in an atmosphere of 7% CO2.

Probes

The probe, 3'α-1.3, a 1.3-kb BamHI/BglII fragment cloned into pSp65, was isolated from the germline phage clone A571.1. This phage clone contains 13.5 kb of the region 3' of the I strain Ca gene (EMBL3 phage vector; a gift of Janet Stavnezer, University of Massachusetts) (19). A Charon phage clone, Ch3'Ca12A (a gift of Kathryn Calame), contained the BALB/c strain Ca germline gene and sequences further 3', including 9.3 kb of the 3'-most EcoRI fragment (20). Subclones derived from Ch3'Ca12A included 3'α-0.5 and 3'α-0.7, both XbaVEcoRI fragments (pUC 18). 3'αE is a 596-bp mouse polymerase chain reaction product (9), which was subcloned into pBSK (Stratagene) at the BamHI and KpnI sites (BamHI and KpnI sites were derived from the ends of the polymerase chain reaction oligonucleotide primers). The probe PstI-2.3 was a gift from Sherie Morrison (21). The probe for the myelin basic protein (22) was a gift from Erik Selsing. All subclones were propagated in the bacterial strain DH5α.

Isolation and analysis of genomic DNA

DNA was prepared from tissue culture cells (23) and liver (24), and digested to completion with an excess of restriction enzyme. Electrophoretic separation of digested DNA was carried out using 10 μg DNA/filament in a 0.8% agarose gel in Tris-phosphate buffer (90 mM Tris-phosphate/2 mM EDTA). The DNA was then transferred to Nytran filters using 20X SSC (Schleicher & Schuell), essentially as described (25). The filters were prehybridized for 2.5 h and then hybridized for 48 to 72 h at 65°C with the appropriate random primed (Stratagene) [32P]dCTP-labeled probe at a sp. act. of 5 X 106 to 3 X 107 cpm/μg and a concentration of 1 X 106 to 5 X 106 cpm/ml. The prehybridization/hybridization buffer contained 5 × Denhardt's/3X SSC/0.1% SDS/10 μg/ml poly A/50 μg/ml salmon sperm DNA. After hybridization, the filters were washed four times (0.1X SSC/0.1% SDS), for 30 min each at 65°C, and subjected to autoradiography at −80°C for variable lengths of time with Kodak RPS-X-Olivet film (Eastman Kodak, Rochester, NY). Plasmid DNA was prepared as described (26).

DNase I assay

Approximately 1 X 106 cells were isolated from each cell line and washed twice with cold PBS. The cells were resuspended at a concentration of 2 X 107 cells/ml in ice-cold Tris buffer (10 mM Tris-HCl, pH 7.8/4 mM MgCl2/1 mM EDTA) and homogenized using a Dounce homogenizer with a B pestle. Cell disruption was monitored with a light microscope. Nuclei were pelleted and resuspended in 20 ml of cold nuclear isolation buffer (NIB, 60 mM KC1/15 mM NaCl/5 mM MgCl2/0.1 mM EDTA/15 mM Tris-HCl, pH 7.4/0.5 mM dithiothreitol/0.1 mM PMSF/0.3 M sucrose) and further purified by overlaying on an equal volume of NIB with 0.6 M sucrose and spun at 1000 rpm for 10 min. Nuclei were resuspended at a concentration of 5 X 107 cells/ml NIB with 5% glycerol. The samples were aliquoted
Results

Location of hybridization probes

The region 3’ of Ca contains three consecutive EcoRI fragments: 5’-12 kb-2 kb-26 kb-3’, with the 3’α enhancer spanning the EcoRI site linking the 12- and 2-kb EcoRI fragments (9, 27) (Fig. 1). The 3’α enhancer is flanked by families of repeat elements. Immediately flanking 3’αE are inverted repeats (10). Further 5’ and 3’—within the 12-kb and 26-kb EcoRI fragments flanking 3’αE—are five BamHI restriction fragments (4.9, 3.3, 1.6, and 1.3 kb, and 885 bp) that hybridize to the 3’α-1.3 probe (27) (Fig. 1). A third repeat family, unique to this region, has also been detected (S. L. Giannini and B. K. Brischtein, unpublished observations). Because of the presence of these repeat families, only a limited number of unique probes are obtainable, and these detect sequences at the 5’ flank of the 12-kb EcoRI fragment (3’α-0.5), within 3’αE (3’αE), and at the 3’ flank of the 26-kb fragment (PstI-2.3).

Methylation patterns

We assessed the methylation status of the 3’α region during B cell development, as represented by a variety of B cell lines. Genomic DNA was initially cut with either HpaII or MspI; both enzymes recognize the sequence CCGG, but HpaII does not digest the DNA if the second cytosine is methylated. Aliquots were then digested with EcoRI and subjected to genomic Southern analysis, initially with the 3’α-1.3 probe. This approach allowed us to examine the relative methylation levels of the 12- and 26-kb EcoRI fragments that contain 3’α-1.3 hybridizing sequences.

Both the 12- and 26-kb EcoRI fragments contain internal MspI sites (Fig. 2). Several MspI sites have been mapped, either directly from restriction cuts of a clone containing the 9.3-kb 5’ segment of the 12-kb EcoRI fragment or from genomic Southern analysis of MspI, EcoRIMspI, and BamHIMspI digests probed with 3’α-1.3, 3’αE, or PstI-2.3 (Fig. 1). A number of these MspI sites are differentially methylated in tissues and cell lines as revealed by differences in restriction patterns between MspI and HpaII. For example, an EcoRIMspI digest of BALB/c genomic DNA, when probed with 3’α-1.3, shows three fragments of 7.6, 6.6, and 1.7 kb (see Figs. 1 and 2, A and B), whereas EcoRIHpaII digestion results in different and higher m.w. bands. A 12-kb EcoRIHpaII fragment present in all tissues and cell lines examined represents the fully methylated 12-kb EcoRI genomic fragment, as shown by hybridization to a specific probe (3’α-0.5) (data not shown).

The largest 3’α-1.3 hybridizing fragment we detected after EcoRIHpaII digestion was 20 kb (see Fig. 2, pre-B cell lines). This fragment clearly arose from the 26-kb EcoRI fragment as a result of at least one constitutively unmethylated CCGG site. We identified two fully unmethylated sites near the 3’ end of the 26-kb EcoRI fragment using a probe specific for this region (PstI-2.3). PstI-2.3 (Fig. 1) hybridized to 1.8 and 1.6 kb EcoRIHpaII and EcoRIMspI fragments in all cell lines tested (Fig. 2C). The most 5’ MspI site detected by PstI-2.3 had previously been detected as an unmethylated site in a myeloma variant, whereas a MspI site 800 bp further 5’ showed partial methylation (21). The two MspI sites at the 3’ end of the 26-kb EcoRI fragment accounted for at least 3.4 kb of the 6 kb lost by HpaII digestion. We then examined the methylation status of the 5’ end of the 26-kb EcoRI fragment. The most 5’ MspI site detected by PstI-2.3 had previously been detected as an unmethylated site in a myeloma variant, whereas a MspI site 800 bp further 5’ showed partial methylation (21). The two MspI sites at the 3’ end of the 26-kb EcoRI fragment accounted for at least 3.4 kb of the 6 kb lost by HpaII digestion. We then examined the methylation status of the 5’ end of the 26-kb EcoRI fragment. This region is spanned by four adjacent BamHI fragments: the fragment order is 5’-6.0 kb-1.3 kb-3.3 kb-4.9 kb-3’ (see Fig. 1). The 1.3- and 4.9-kb 3’α-1.3 hybridizing BamHI...
FIGURE 2. Methylation patterns in the region 3′ of Ca. Ten micrograms of DNA derived from BALB/c spleen, liver, and brain (A), or lymphoid cell lines (B, C, and D) were cut with MspI (M) or HpaII (H) and then with EcoRI (E) (A, B, and C) or with BamHI (D). Samples were run on an 0.8% agarose gel before Southern blot analysis with 3α-1.3 (A, B, and D) or Psf-2.3 (C) as hybridization probes. See Materials and Methods for identification of cell lines and Figure 1 for location of hybridization probes. In addition to 26-kb and 12-kb 3′α-1.3 hybridizing EcoRI fragments, rarely we have observed other hybridizing bands and then only at high DNA concentrations (see, e.g., BCL1 and MPC11, B). The additional 3′α-1.3 hybridizing MspI fragment in S107 (B) derives from a previously observed 3′α-rearrangement (27). The hybridization pattern for SW5147 (D) reflects its AKR/J origin.

fragments contain CCGG sites, as shown by BamHI/MspI digestion, but are fully methylated in cell lines that contain the 20-kb EcoRI/HpaII fragment (cell lines 3′-1 and 18-81 in Fig. 2D). We detected one MspI site in the 6.0-kb BamHI fragment, which showed a similar methylation pattern (data not shown). Based on the presence of fully methylated sequences at the 5′ end of the 26-kb EcoRI fragment in cell lines that contain the 20-kb EcoRI/HpaII fragment, we conclude that constitutively unmethylated MspI sites are located at the 3′ end of the 26-kb EcoRI fragment, commencing ~21.5 kb 3′ of 3′αE.

In addition to the constitutively unmethylated sites at the 3′ end, we observed differential methylation of the 5′ 20-kb segment of the 26-kb EcoRI fragment. In nonlymphoid organs, such as liver and brain (and kidney; data not shown), the 20-kb EcoRI/HpaII fragment was only partially methylated, as evidenced by smaller EcoRI/HpaII fragments, in addition to the predominant 12-kb and fainter 20-kb EcoRI/HpaII cleavage products (Fig. 2A). In the spleen (Fig. 2A) and in T cell lines (Fig. 2A and 2D), increased methylation of the 20-kb band was observed. In spleen cells (Fig. 2A), in addition to 20- and 12-kb EcoRI/HpaII fragments, we detected the presence of 6.6- and 5.2-kb fragments. As indicated, bands at these positions were also present in other sources (see, for example, faint bands in liver and stronger bands in myeloma cells). The 6.6-kb EcoRI/HpaII fragment is identical in size to a 6.6-kb EcoRI/MspI fragment, resulting from cleavage at a partially demethylated MspI site in a 1.6-kb BamHI fragment located in the 12-kb EcoRI fragment (see Fig. 1). The 5.2-kb fragment necessarily reflects partial methylation at the same site and at MspI/HpaII sites further upstream. The immediate upstream MspI site either is totally methylated or undergoes reciprocal methylation with the site in the 1.6-kb BamHI fragment, since cleavage at these adjacent MspI sites—were they both undermethylated—would have revealed a 1.7-kb HpaII fragment, which was not observed. We thus conclude that undermethylation occurs in a punctuated fashion in this region.

Early in B cell differentiation, the entire 20-kb EcoRI/HpaII fragment was hypermethylated: in pre-B cell lines (Fig. 2B) (18-81 and 3′-1), only a 20-kb fragment (in addition to the fully methylated 12-kb fragment) was detected with 3′α-1.3. In B cell lines (Fig. 2B), (BCL1 and W231),5 the 20-kb EcoRI/HpaII fragment was also generally hy-
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FIGURE 3. Localization of DNAse I-hypersensitive sites in the region 3′ of Ca. Nuclei from cell lines were treated with varying quantities of DNAse I for 3 min before the isolation of DNA, as described in Materials and Methods. Ten micrograms of purified DNA were digested with EcoRI (A) or BamHI (B) before Southern blots, using as probes 3′α-1.3 (A) or 3′αE (B). The quantity of DNAse I used is indicated at the top of each lane. The hypersensitive bands are indicated by asterisks. See map in Figure 1 for locations of hypersensitive sites. A 2.1-kb BamHI fragment that faintly hybridizes to 3′α-1.3 is shown in Figure 3B. This fragment is located between 1.6-kb and 885-bp BamHI fragments (Fig. 1).

permethylated, although in some samples we detected partial demethylation, as represented by diminution of the signal of the 20-kb fragment and the appearance of smaller restriction fragments. In plasma cells, as represented by several myeloma cell lines synthesizing various H chain isotypes (Fig. 2B; TEPC 1017 (IgD), NSO (IgG1), MPC I1 (IgG2b), and S107 (IgA)), the 20-kb EcoRI/HpaII fragment was no longer detectable, consistent with considerable undermethylation of this region.

Thus, although the region 3′ of 3′αE was partially methylated in non-B cells, both the pre-B and B cell stage were marked by hypermethylation. Extensive demethylation occurred late in B cell differentiation at the plasma cell stage, and affected regions both 5′ and 3′ of 3′αE.

DNAse I hypersensitivity

To assess the DNAse I hypersensitivity pattern of the ~40-kb region in which 3′αE is located, nuclei were isolated from pre-B, myeloma, and T cell lines and treated with DNAse I. After extraction, the DNA was digested with EcoRI and analyzed by Southern blots, using the 3′α-1.3 probe (Fig. 3A). In the absence of any hypersensitive sites, we expected to detect restriction fragments of 26 and 12 kb; any additional bands would indicate hypersensitive sites. In pre-B cells, a prominent sub-band of 15 kb was observed, whereas in myeloma cells a prominent sub-band of 10 kb was observed. In some preparations from myeloma cells, the 15-kb sub-band was also present. In the T cell line EL-4, there were no hypersensitive sites, and the region as a whole was more resistant to DNAse I. As a negative control in these experiments, we used a probe for the myelin basic protein gene (22). EcoRI-digested DNA probed for the myelin basic protein gene did not display any hypersensitive sites (data not shown). These results indicate that the hypersensitive sites within the 3′α region are present at defined stages of B cell development.

Our mapping data of the region allowed us to localize the hypersensitive sites. The 15-kb hypersensitive fragment, detected in pre-B cell lines, was necessarily derived from the 26-kb EcoRI fragment. Since all the 3′α-1.3 hybridizing sequences reside within a 9.5-kb region at the 5′ end of the 26-kb EcoRI fragment and only one sub-band (15 kb) was detected with this probe, the hypersensitive site is most
likely located 15 kb from the 5’ end of the 26-kb fragment (see Fig. 1, arrow). Experiments to clone this segment are in progress.

The 10-kb hypersensitive band detected in myeloma cells could have derived from either the 12- or 26-kb EcoRI fragment. We therefore located this site by digesting DNAse I-treated DNA with BamHI (Fig. 3B). No sub-bands were detected with the 3’α-1.3 probe, implying that hypersensitive sites were not present in the five 3’α-1.3-hybridizing BamHI fragments and that other probes were needed to map the site(s). Examination of the 3’ end of the 12-kb fragment was carried out with the probes 3’α-0.7 or 3’αE, which detect a 6.0-kb BamHI fragment (Figs. 1 and 3). Two hypersensitive sites were detected, located 2.5 and 2.2 kb 3’ of the 5’ BamHI site (3’α-0.7), or reciprocally, 3.8 kb (6.0 to 2.2 kb) upstream of the 3’ BamHI site (3’αE) (Fig. 1, arrows). Thus, in myeloma cells, a pair of DNAse I-hypersensitive sites is located within 500 bp 3’ of 3’αE, one of which (2.5 kb 3’ of BamHI) appears to lie within the region that contains a repeated part of the 3’α enhancer (10). The mapping of these hypersensitive sites in myeloma B cells within or very close to 3’αE may be indicative of enhancer function at this late stage of B cell development.

**Discussion**

Regions flanking the 3’α enhancer showed cell typespecific variations in methylation and DNAse Ihypersensitive sites, as summarized in Table I. In both nonlymphoid and T cell lines, sequences between Cα and 3’αE were hypermethylated (the 12-kb EcoRI fragment remained uncut by HpaII). In nonlymphoid cells, the region 3’ of 3’αE was considerably undermethylated, whereas in T cells, it was hypermethylated. In all sources (including B cell lines), a region of constitutively undermethylated DNA lay further 3’-commencing ~21.5 kb 3’ of 3’αE. No DNAse I-hypersensitive sites were detected in T cell lines.

In cell lines representing progressive stages of B cell differentiation, there was considerable change in the methylation status of sequences flanking 3’αE. In pre-B and B cell lines, as in nonlymphoid cells and T cells, the 12-kb EcoRI fragment was maintained in a predominantly hypermethylated state; however, the region 3’ of the enhancer showed extensive changes. In pre-B cell lines (as in T cell lines), most of this 3’ flanking region was hypermethylated, as evidenced by a single prominent EcoRI-HpaII fragment of 20 kb. A DNAse I-hypersensitive site located approximately 17.0 kb 3’ of 3’αE was present in pre-B cell lines and may indicate a regulatory element operative during an early stage of B cell development. Thus, commitment to both B and T lymphoid lineages was evidenced by hypomethylation of the region 3’ of 3’αE. In T cells, the hypomethylation of the 3’α region may be part of a specific mechanism to silence the IgCl locus. Pre-B cells were distinguished from T cells by an acquisition of a DNAse I-hypersensitive site in this segment.

Although a loss of methylated sites in the region 3’ of 3’αE was occasionally evident in some samples of B cell lines, it was at the plasma cell stage of development, and independent of the isotype of the expressed H chain, that the 20-kb EcoRI/HpaII fragment became completely undermethylated at several sites so that it was no longer detectable. Undermethylation also affected the 12-kb EcoRI fragment. However, the 12-kb EcoRI fragment never became fully undermethylated since the EcoRI/HpaII and EcoRI/MspI cleavage patterns remained different and some 12-kb signal was always present. At this late stage in B cell development, additional DNAse I-hypersensitive sites were also observed within or near an inverted repeat region 5’ of 3’αE. The observation of DNAse I hypersensitivity in close proximity to 3’αE in plasma cells provides intriguing insight into the potential timing of action of this regulatory element during B cell differentiation.

We identified a differentially methylated site within the 4.9-kb 3’α-1.3 hybridizing BamHI fragment from the 26-kb EcoRI fragment (Fig. 2D). This site was highly methylated in pre-B cells (3-1 and 18–81), B cells (BCL-1), and T cells (BW5147) and undermethylated in myeloma cells (TEPC1017, NSO, and MPC11) (Fig. 2D). Differential methylation of a MspI site within the 1.3-kb BamHI fragment from the 26-kb EcoRI fragment was not easy to show directly because of the small differences in sizes of the cleaved and uncleaved fragments. However, it is likely that this site became undermethylated in plasma cells since a 7.6-kb 3’α-1.3 hybridizing HpaII fragment that would result from cleavage at this position could be detected. As stated above, differential methylation of the 6-kb BamHI fragment that contains 3’αE was not evident (data not shown). Thus, the predominant region of differential methylation spanned ~19 kb commencing ~2 kb 3’ of 3’αE and ending ~21.5 kb 3’ of 3’αE.

Despite the presence in pre-B cell lines of a DNAse I-hypersensitive site ~17 kb 3’ of 3’αE, our methylation data
suggest that 3'αE itself is situated in an inaccessible chromosomal conformation. Other studies from our laboratory have identified a B lineage-specific nuclear DNA binding protein, NFHβ, that binds to several sites within and flanking 3'αE, including 3'α-885 and 3'α-1.6 (Fig. 1) (28) (M. Singh and B. K. Birshtein, manuscript in preparation). NFHβ is the most likely identical to SoBP (29) and BSAP (30), the latter a B cell transcription factor, which has recently been shown to be important for V pre-B (31), λ5 (31), and CD19 (32) gene expression. This DNA binding protein is present only during early stages of B cell differentiation, namely pro-B, pre-B and B cells, which are the same stages during which considerable methylation of the 26-kb EcoRI fragment 3' of the enhancer was detected (see Table I). It is intriguing to consider that the relative inaccessibility of the enhancer at these early stages of B cell differentiation may be mediated by the presence of this specific DNA binding protein. Sequences flanking enhancers of various tissue-specific or developmentally regulated genes have been observed to confer repression (33–39). Furthermore, methylated CpG-specific DNA binding proteins (MeCP) have recently been identified in vitro and in vivo assay systems. These proteins bind to DNA methylated at CpGs, irrespective of the surrounding sequence composition (40–42). Such proteins may interact at hypermethylated regions flanking 3'αE and thereby contribute to its sequestration.

We suggest that upon B cell activation, the accessibility of the 3'α region is altered, thus allowing the 3'α enhancer to exert its effect. Such a correlation of variation in methylation with developmentally regulated gene expression has been previously demonstrated (43, 44). Interestingly, in transient transfection assays, 3'αE was reported to be active only during the later stages of B cell development, in myeloma cells (10). This is the same stage in which DNase I-hypersensitive sites are present in close proximity to 3'αE. Furthermore, Eμ-independent IgH expression was detected only in cell lines representing these same late stages.

A number of important steps in Ig gene expression occur at the B → plasma cell stage of differentiation, the time during which 3'αE may become operative. These include H chain class switching and somatic hypermutation of VH and VL genes, in addition to a shift to high levels of secreted antibody production. As 3'αE becomes accessible at later stages of B cell activation and differentiation, structural interactions may take place between this element and promoters within the IgH gene cluster. As a prerequisite to class switching, germline transcripts of particular Cγ genes are detectable (45–48). The promoters for these transcripts lie upstream of individual Cγ genes within the IgH gene cluster, thereby significantly closer to 3'αE than is the expressed VH promoter. Furthermore, as a result of deletions associated with class switching, the distance between the expressed VH promoter and 3'αE, although still substantial, may be significantly reduced. In this respect, we have observed a ∼70-kb inversion of the expressed H chain cluster in the IgG2b-producing mouse myeloma cell line F5.5 (27). This inversion appears to have resulted from resolution of a loop structure, anchored by the physical interaction of the 3'α region with VH sequences at the 5' end of the Ig cluster.

Interestingly, a region 3' of the Cκ gene, which includes the 3'κ enhancer, has been inferred to be required for somatic hypermutation of transgenic κL chain genes (49). Whether the region 3' of Cκ is analogously implicated for VH somatic hypermutation is not yet known.

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