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Methylation status and DNase I sensitivity of immunoglobulin genes: Changes associated with rearrangement

(transcriptional regulation/κ locus/chromosomal domains)

ELIZABETH L. MATHER* AND ROBERT P. PERRY

Institute for Cancer Research, Fox Chase Cancer Center, Philadelphia, Pennsylvania 19111

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ABSTRACT **Immunoglobulin V_κ genes are transcriptionally silent in their germline context and become transcriptionally active upon fusion to the J_κ–C_κ region (κ locus). To elucidate the role of chromosomal structure in this regulatory phenomenon we have investigated the DNase I sensitivity and methylation status of the κ locus and selected V_κ genes in a variety of alleles exhibiting different rearrangement configurations and different levels of transcriptional activity. Our findings indicate that the κ locus in either germline or rearranged contexts maintains a distinctive DNase I-sensitive, hypomethylated structure in plasmacytomas and hybridomas, irrespective of its level of transcriptional activity. In contrast, the germline V_κ genes are in less accessible regions of chromatin and more highly methylated regions of DNA. Upon fusion to the κ locus, V_κ genes become DNase I-sensitive and hypomethylated. This effect extends several kilobases upstream of the transcriptional initiation site but does not extend to the adjacent V_κ gene or to the identical V_κ allele on the other chromosome, indicating that the structural alteration is a localized *cis*-acting phenomenon.**

The mechanisms by which specific genes are selectively expressed in differentiated cells are poorly understood. One of the critical factors in gene expression appears to be chromatin structure; transcriptionally active genes are largely confined to chromatin domains in which the DNA structure is in a relatively extended or open configuration. This open configuration is characterized by increased sensitivity to DNase I digestion, DNA hypomethylation, and possibly by differential binding of chromosomal proteins (1, 2). Little is known about how this open configuration arises or how it is maintained.

The immunoglobulin genes are particularly useful for studying this problem. Because they undergo novel somatic rearrangements on one or both allelic chromosomes, they offer a unique situation in which identical or nearly identical genetic elements exist in entirely different chromosomal contexts and have markedly different levels of transcriptional activity within the same nucleus. These genes thus provide one with an ideal system for examining the relationship between the context of a gene—e.g., its proximity to putative regulatory elements (3–5)—and its higher order structure, as revealed by DNase I sensitivity and extent of DNA methylation.

The gene encoding the κ light chain is formed by a DNA rearrangement that brings together one of many V_κ gene segments and the J_κ–C_κ gene segment (6, 7). Although such a rearrangement is required to form a functional (κ^+) gene, it is not a prerequisite for transcriptional activity at the locus. The C_κ gene is transcriptionally competent even in the unrearranged or germline (κ^0) configuration, as demonstrated by the nuclear transcripts in plasmacytomas and pre-B cell hybridomas that initiate at a site on the κ^0 allele about 8 kilobases (kb) upstream of the C_κ exon (8–10). In contrast, the V_κ genes are transcriptionally silent in their germline context (11) and acquire transcriptional competence only when fused to the J_κ–C_κ segment. The rate of transcription of a κ^0 allele, although generally lower than that of a rearranged κ gene (10), is still relatively high compared to most other cellular genes.

In line with the results obtained from studies of other genes, the transcriptionally active, rearranged κ genes and the germline κ^0 gene are more sensitive to DNase I digestion than the germline V_κ genes (12, 13). These results raise several important questions. What happens to the chromatin structure of a V_κ gene when it is rearranged? How are neighboring V_κ genes affected? Is there a difference in the extent of DNA methylation of unrearranged and rearranged C_κ and V_κ genes? How is the extent of DNA methylation related to the transcriptional activity of the locus? The experiments reported here were designed to answer these questions and, as a result, to provide new insight into the mechanisms of gene regulation.

MATERIALS AND METHODS

DNA extracted from cultured lines of MPC 11, 15-17-6 (H796), and X63-Ag8.653 cells (9, 14), from a variety of mouse plasmacytomas (8), from mouse embryos, and from adult mouse liver was analyzed by the Southern blot procedure as described (15). DNA samples were digested with two 15-min units of restriction enzyme (New England BioLabs) per μg for 3 hr at 37°C. The hybridization probes were as follows: (*i*) a gel-purified 2.9-kb *Bam*HI–*Msp* I fragment containing V_κ19A and 5′ flanking sequences, prepared from a genomic clone of the expressed gene of MPC 11 cells (16); (*ii*) a cDNA clone from MPC 11 cells, pκ(11)[24] (14), containing V_κ19A + C_κ sequences; (*iii*) and (*iv*) subcloned fragments containing V_κ21C and C_κ sequences, respectively, derived from a MOPC 321 cDNA clone (8); and (*v*) a 6.3-kb *Eco*RI–*Bam*HI fragment spanning the J_κ–C_κ region (pEC$_\kappa$) derived from a cloned embryonic C_κ gene (17). For the DNase I sensitivity studies, MPC 11 nuclei were isolated as described (18), resuspended in 0.01 M Tris·HCl, pH 7.4/0.01 M NaCl/3 mM $MgCl_2$ to 1.4×10^8 nuclei per ml, and incubated with 0–7.5 μg of DNase I (Worthington) per ml for 1 min at 26°C. The reaction was stopped by the addition of EDTA to 50 mM and sodium dodecyl sulfate to 1%.

RESULTS

DNase I Sensitivity of V_κ Genes Depends on Their Chromosomal Location. The myeloma cell line MPC 11 was selected for analysis because the genetic organization and transcriptional activity of its κ genes have been well characterized. In this line both κ alleles are rearranged and transcribed at a similar rate

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Abbreviations: kb, kilobase(s); V, variable.

* Present address: La Jolla Cancer Res. Found., La Jolla, CA 92037.

(14). On the κ^+ allele a member of the V_κ19 family (denoted V_κ19A) is correctly fused to the J_κ2 element to form a gene that specifies a proper κ chain (16). On the other (κ^-) allele a member of the V_κ21 family (V_κ21C) is abnormally rearranged to a site within the J_κ–C_κ intron (19). Transcripts of the κ^- allele are aberrantly processed to yield mRNAs lacking variable (V) region sequences (8, 20). The V_κ21 genes are located downstream from the V_κ19 family and are deleted from the κ^+ allele (21). In contrast, all of the V_κ19 genes are retained on the κ^- allele, as are the members of the V_κ21 family that lie upstream from V_κ21C. Thus, in addition to the rearranged V_κ19 and V_κ21 genes, MPC 11 cells contain at least one full set of V_κ19 genes and a partial set of V_κ21 genes in their germline context. Because the linkage of several V_κ21 genes is known (22), some idea of the size of the DNase I-sensitive domain can be obtained by comparing the sensitivity of the residual V_κ21 germline genes with that of the rearranged V_κ21C gene.

DNA isolated from MPC 11 nuclei that had been treated with various concentrations of DNase I was digested with *Bam*HI or *Eco*RI and analyzed by Southern blotting. Our ability to discriminate between active and inactive genes with this protocol was verified by comparing the sensitivity of the C_κ genes with that of the α-fetoprotein gene, which is not expressed in these cells (Fig. 1*A*). Whereas the C_κ-containing *Bam*HI fragments of 2.9 and 8 kb are barely detectable after digestion with 2.5 μg of DNase I per ml, there is a significant residuum of a 3.0-kb fragment containing the α-fetoprotein gene (23) after digestion with a 3-fold higher concentration of DNase. When a similar blot of *Eco*RI-digested DNA was hybridized with a cDNA probe that reveals both V_κ19 and C_κ sequences (Fig. 1*B*), we observed that the V_κ19 genes in germline context, including the V_κ19A gene (8.7-kb fragment) and a closely related member, V_κ19B (6.0-kb fragment), were also relatively resistant to DNase I digestion compared to the expressed V_κ19A–C_κ gene contained within the 21-kb fragment. Similarly, when a probe specific for V_κ21 sequences was hybridized to an *Eco*RI-digested series (Fig. 1*C*), the 20-kb fragment containing the transcribed gene exhibited high sensitivity, whereas the V_κ21 genes in germline context are relatively resistant. Although some of the DNase I sensitivity of the 20- to 21-kb *Eco*RI fragments might be attributed to their relatively large target size, it is clear from the equivalent sensitivity of the considerably smaller *Bam*HI fragments containing the same genes (Fig. 1*A*) that size is not the principal determinant of this sensitivity. The DNase I resistance of the 6.3-kb V_κ21 fragment is particularly significant because this fragment is known to contain the 5′ nearest neighbor of V_κ21C, separated from it by about 21 kb (22). Thus, the domain of DNase I sensitivity does not extend to the adjacent V_κ gene of this familial cluster.

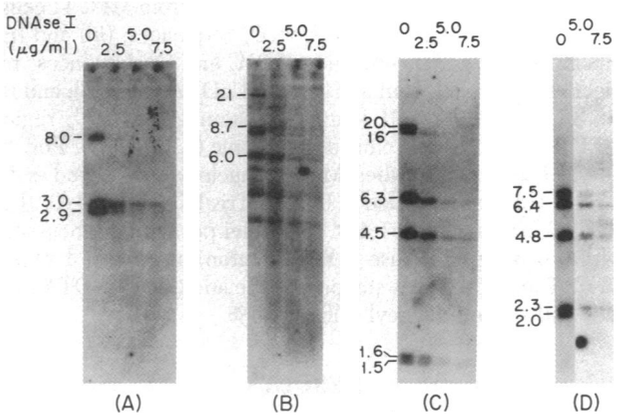

FIG. 1. DNase I sensitivity of rearranged and germline V_κ genes. MPC 11 nuclei were treated with 0–7.5 μg of DNase I per ml, the DNA was isolated and digested with restriction enzyme, and various genes were identified by Southern blot analysis. (*A*) *Bam*HI digest, hybridization probes *iv* (C_κ) and a genomic fragment specific for the α-fetoprotein structural gene. (*B*) *Eco*RI digest, hybridization probe *ii* (V_κ19 and C_κ). (*C*) *Eco*RI digest, hybridization probe *iii* (V_κ21). (*D*) *Bam*HI digest, hybridization probe *iii*. Fragment sizes in kb are indicated.

To determine whether the DNase I-sensitive property of the C_κ locus is actually conferred upon the incoming V_κ gene it is necessary to analyze a restriction fragment that uniquely comprises V_κ sequences and that does not encompass the J_κ–C_κ portion of the rearranged gene. Because the foregoing experiments as well as those presented in earlier studies (12, 13) utilized fragments spanning both V_κ and J_κ–C_κ sequences, they do not critically address this issue. Fortunately, several members of the V_κ21 family, including V_κ21C, have a *Bam*HI site within the V coding region so that with a *Bam*HI digest and a V_κ21-specific probe one can readily analyze only the V-containing portion of the rearranged gene. An experiment of this type (Fig. 1*D*) clearly demonstrated that the rearranged V_κ21C gene (2.0-kb fragment) does indeed acquire the DNase I-sensitive characteristic of the C_κ locus.

Methylation Status of V_κ and C_κ Genes in MPC 11 Cells and Liver. Several restriction enzyme sites containing CpG doublets were used as indicators of methylation status. The enzymes employed were *Hha* I, which recognizes the sequence G-C-G-C but not G-Cm-G-C; *Hpa* II, which recognizes C-C-G-G but not C-Cm-G-G; and its isoschizomer *Msp* I, which recognizes both C-C-G-G and C-Cm-G-G. Three *Hha* I sites in the J_κ region and a *Hpa* II/*Msp* I site within the V_κ19A gene were known from sequence data (16, 24); the other sites were identified during the course of the analysis. We confined our studies mainly to the category of *Hha* I or *Hpa* II/*Msp* I site that exhibits variable methylation with respect to tissue type. Several sites that were found to be largely unmethylated both in lymphoid cells and in a nonexpressing tissue like liver are not discussed because they are unlikely to be indicators of functionally important changes in DNA.

When the methylation status of the three sites within the 8.1-kb *Bam*HI fragment spanning the κ^+ allele was examined (Fig. 2*A*), all were found to be unmethylated, as evidenced by

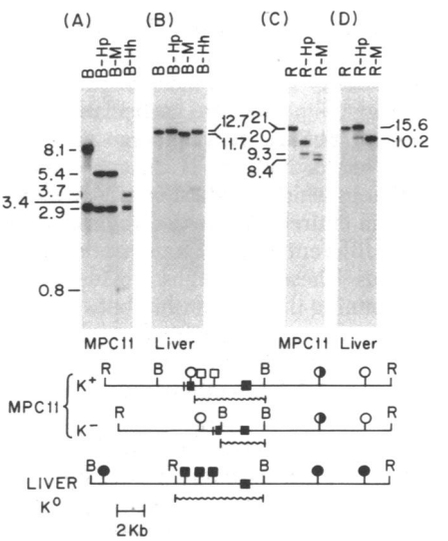

FIG. 2. Methylation status of κ loci in MPC 11 cells and mouse liver. DNA was digested with either *Bam*HI (B) or *Eco*RI (R) in combination with either *Hpa* II (Hp), *Msp* I (M), or *Hha* I (Hh), blotted, and hybridized with probe *v* (J_κ–C_κ). In the schematic diagram, circles refer to *Hpa* II/*Msp* I sites, and boxes refer to *Hha* I sites. The symbols are filled if >90% of the sites are methylated, open if <10% are methylated, and half-filled if the extent of methylation falls within these limits. The wavy lines show the extent of overlap with the probe.

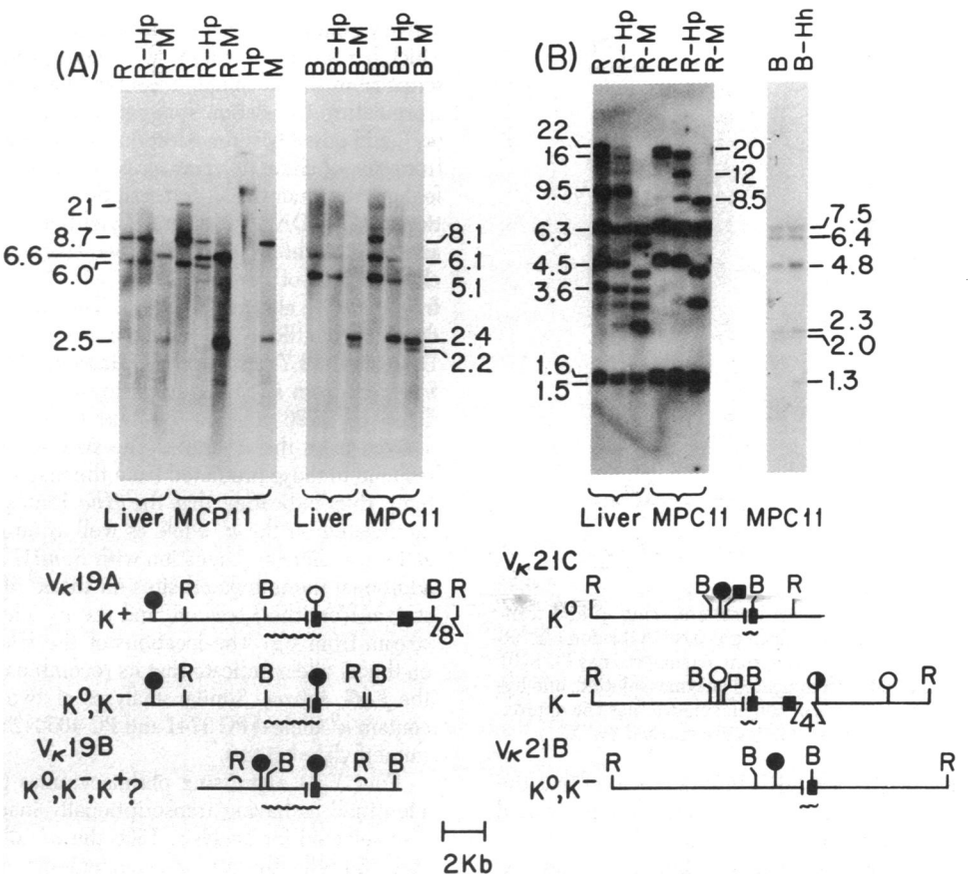

FIG. 3. Methylation status of the V_κ19 and V_κ21 genes in MPC 11 cells and liver. Digestions as in Fig. 2 hybridized with V_κ19 probe *i* (*A*) or V_κ21 probe *iii* (*B*). In the schematic diagrams the *V* genes are shown as they occur on different chromosomal alleles—e.g., V_κ19A is shown both in rearranged (κ^+ of MPC 11 cells) and germline (κ^- of MPC 11 cells or κ^0 of liver) contexts. For convenience the maps of V_κ19A (κ^+) and V_κ21C (κ^-) were shortened by 8 and 4 kb, respectively, at the positions indicated. The positions of the *Eco*RI sites in V_κ19B are not known precisely, as indicated by the small arcs.

the complete loss of this fragment upon digestion with *Bam*HI and either *Hpa* II or *Hha* I and by the appearance of product fragments of appropriate size. The fact that the *Bam*HI/*Hha* I digest produces novel fragments of about 3.7, 3.4, and 0.8 kb indicates that neither *Hha* I site is methylated on the κ^+ allele. No sites are contained within the 2.9-kb κ^- *Bam*HI fragment and therefore this fragment remained unchanged during the subsequent digestions with *Hpa* II or *Hha* I. These same *Hha* I sites are almost completely methylated in liver DNA as are an additional *Hha* I site and a *Hpa* II/*Msp* I site located 0.1 and about 6 kb upstream of J_1, respectively (Fig. 2*B*). From the sizes and intensities of the faint bands seen in the *Bam*HI–*Hha* I digest of liver DNA we estimate that <10% of the liver DNA molecules lack methyl groups at any of the three *Hha* I sites and that essentially none lacks methyl groups at more than one site. The *Hpa* II/*Msp* I site internal to the V_κ19A gene is also fully methylated in liver DNA (Fig. 3*A*).

Additional methylation sites were examined by determining the effect of *Hpa* II or *Msp* I digestion on the relatively large *Eco*RI fragments encompassing the κ alleles (Fig. 2*C*). Although the large (20–21 kb) *Eco*RI fragments containing the κ^+ and κ^- alleles are incompletely resolved, double-digestion with *Eco*RI and *Msp* I or digestion with *Msp* I alone yielded two discrete fragments of 9.3 and 8.4 kb. These fragments encompass the κ^+ and κ^- alleles, respectively, as ascertained by comparison with a blot hybridized with the V_κ21-specific probe (cf. Fig. 3). Digestion with *Eco*RI and *Hpa* II yielded the same two fragments, an additional pair about 12.8 and 11.9 kb, and a small residuum of the 20-kb κ^- fragment. These results enabled us to map three additional *Hpa* II/*Msp* I sites: an unmethylated site on the κ^- allele about 1.1 kb upstream of the V_κ21C gene, a partially (25–50%) methylated site, M_1, about 5 kb downstream of the C_κ gene on both alleles, and a third site, M_2, about 3.4 kb further downstream. Only about 10% of the genes that are methlyated at M_1 are also methylated at M_2. In marked contrast, sites M_1 and M_2 are both methylated in >90% of liver DNA molecules (Fig. 2*D*), as is the site upstream of the V_κ21C gene, indicated by the *Hpa* II resistance of the 9.5-kb *Eco*RI fragment containing the V_κ21C germline gene (Fig. 3*B*). The foregoing results, schematically diagramed in Fig. 2, demonstrate that the κ locus is relatively hypomethylated when it is actively transcribed, a property shared by many other cellular genes (2).

We next compared the methylation status of the rearranged and germline V_κ genes in MPC 11 cells. To simplify the analysis of the V_κ19 family we used a probe of genomic origin which is mainly composed of 5′ flanking sequence of V_κ19A; with brief exposures of the autoradiographs this probe detects only the V_κ19A gene and its close relative, V_κ19B. The data of Fig. 3*A*, together with previous analyses, enabled us to map the V_κ19A and V_κ19B genes and to identify two additional *Hpa* II/*Msp* I sites besides the one in the V region. When in the germline context all of these sites are highly methylated in MPC 11 cells as well as in liver. This is shown by the inability of *Hpa* II to cleave the germline V_κ19A-containing fragments (8.7-kb *Eco*RI; 5.1-kb *Bam*HI) or the germline V_κ19B-containing fragments (6.0-kb *Eco*RI; 6.1-kb *Bam*HI). In agreement with the data of Fig. 2, the 21-kb *Eco*RI and the 8.1-kb *Bam*HI fragments containing the rearranged V_κ19A gene were again readily cleaved by *Hpa* II at the internal site to yield the appropriate sized products

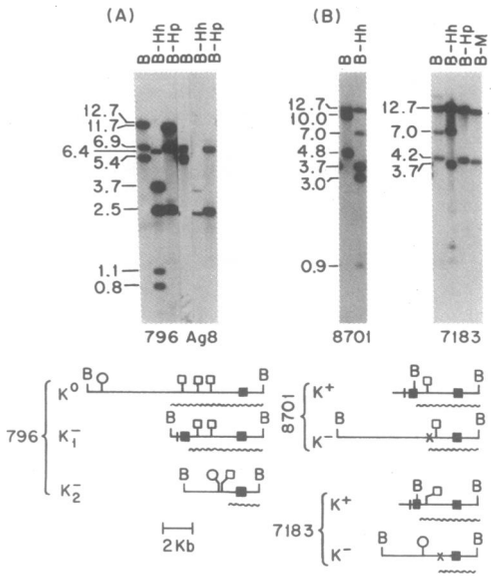

FIG. 4. Methylation status of κ loci in cells bearing κ^0 and transcriptionally inactive κ^- alleles. DNA from pre-B cell hybridoma H796 and its myeloma parent Ag8.653 (*A*) and from plasmacytomas PC 8701 and PC 7183 (*B*) was digested with indicated enzymes, blotted, and hybridized with probe *iv*. In the maps of the plasmacytomas the approximate recombination sites on the κ^- alleles are marked by "X".

[6.6 kb (*Eco*RI) and 2.4 kb (*Bam*HI)], thus confirming that the rearranged copy of the V_κ19A gene is completely unmethylated at this site. However, as seen by comparative *Hpa* II/*Msp* I single digests, the property of hypomethylation does not extend to the 5′ proximal site, which is located about 8 kb further upstream.

Similar results were obtained when the V_κ21 genes in MPC 11 cells were analyzed (Fig. 3*B*). Methylation sites were identified in the 5′ flank of the V_κ21C gene and in two of the V_κ21 genes that remain in the germline context in these cells—namely, V_κ21B and V_κ21A—contained within 16.0- and 4.5-kb *Eco*RI fragments, respectively. Both germline V_κ21 genes are fully methylated, as indicated by the inability of *Hpa* II to cleave the 16.0- and 4.5-kb *Eco*RI fragments. Conversely, the rearranged V_κ21C gene is undermethylated in regions both upstream and downstream of the abnormal recombination site. This is seen by the *Hpa* II cleavage of the 20-kb (κ^-) *Eco*RI fragment to yield products of 8.5 and 12.0 kb or by the *Hha* I cleavage of the 2.0-kb *Bam*HI fragment to yield a product of 1.3 kb. In liver all of the V_κ21 germline genes except those on the large 22-kb *Eco*RI fragment were in regions of DNA containing fully methylated *Hpa* II sites. These results indicate that the state of undermethylation, like that of DNase I sensitivity, is acquired only by the V_κ gene fused to the C_κ locus; identical V_κ genes on the other chromosome or closely related ones further upstream on the same chromosome remain in the highly methylated state characteristic of nonexpressing tissue.

Undermethylation of the C_κ Region in Relation to Chromosomal Context and Transcriptional Activity. The fact that both the κ^+ and κ^- alleles are undermethylated in MPC 11 cells is not surprising because both alleles are transcribed at comparably high rates. To determine whether this condition of hypomethylation would also apply to the κ^0 locus, which is usually transcribed less efficiently than κ^+ loci (10), or to a κ^- locus that is known to be transcriptionally inactive, we examined the methylation status of the C_κ region in a variety of lymphoid tumor lines in which the configuration of the κ loci and their transcriptional activity are known.

One of the lines studied is the pre-B cell hybridoma H796, which contains a κ^0 allele of fetal liver origin and two κ^- alleles derived from the myeloma parent Ag8.653, a nonproducing line which has lost its κ^+ allele. H796 cells produce an 8.4-kb transcript from the κ^0 allele, a nonfunctional κ-mRNA containing a premature translation stop codon from one of the κ^- alleles (κ_1^-), and possibly some additional components (2.3 and 2.7 kb) from the κ_2^- allele (9). Transcription of the κ^- alleles is 3- to 6-fold greater than the κ^0 transcription in these cells (10). Digestion of H796 DNA with *Bam*HI yields a 12.7-kb fragment characteristic of the κ^0 allele and two fragments (6.9 and 5.4 kb) characteristic of the Ag8.653 κ^- alleles (Fig. 4*A*). All of these fragments are cleaved by *Hha* I. The *Hha* I digestion products derived from the κ^- alleles, seen in a parallel digest of Ag8.653 DNA, are a 3.7- and a 0.8-kb fragment from κ_1^- and a 2.5-kb fragment from κ_2^-. In addition to these components the *Hha* I digest of H796 yields a 6.4- and 1.1-kb fragment, which are derived from the κ^0 allele. The sizes of these fragments correspond to those predicted from the map positions (Fig. 4, bottom), thus indicating that the *Hha* I sites are completely unmethylated on the κ^0 allele as well as on the transcriptionally active κ^- allele(s). Digestion with *Bam*HI and *Hpa* II revealed additional unmethylated sites on the κ^0 allele (about 6 kb upstream from the J_κ region) and the κ_2^- allele (about 1.4 kb upstream from C_κ). The locations of the *Hha* I and *Hpa* II sites on the κ_2^- allele indicate that its recombinational site lies within the J_κ–C_κ intron. Similar analyses of two plasmacytomas that contain κ^0 alleles [PC 3741 and PC 4050 (25)] also revealed their unmethylated status.

Four V_κ21 expressing plasmacytomas that were previously identified as having transcriptionally inactive κ^- genes were also selected for analysis. Like the κ^- alleles in MPC 11 and Ag8.653 cells, these κ^- rearrangements involve fusions to sites within the J_κ–C_κ intron rather than to a J_κ segment. However, in contrast to MPC 11 and Ag8.653, this group of plasmacytomas gave no evidence of producing RNA transcripts other than those related to the authentic V_κ21-mRNA (25). A more detailed analysis of one of these tumor lines, PC 7183, showed that the κ^- rearrangement involves a chromosomal translocation that generates a κ^- allele lacking a *V* gene element and its associated transcriptional initiation signal (26). The analyses of the methylation status of the κ alleles in two of these plasmacytomas are shown in Fig. 4*B*. In PC 8701 the 4.8-kb κ^+ and the 10.0-kb κ^- *Bam*HI fragments are both cleaved by *Hha* I, yielding a 3.7-kb fragment from the κ^+ allele and a 3.0- and a 7.0 kb fragment from the κ^- allele. Thus, both the κ^+ allele and the κ^- allele are largely undermethylated in these cells. In PC 7183 the 4.2-kb *Bam*HI fragment containing the κ^+ allele is cleaved by *Hha* I and the 7.0-kb κ^- fragment is cleaved by *Hpa* II, again indicating unmethylated sites on both alleles. As indicated by the presence of the 12.7-kb *Bam*HI fragment, both of these tumor DNA preparations contain some κ^0 allele presumably contributed by contaminating nonlymphoid cells. As might be expected, κ^0 alleles of nonlymphoid origin are fully methylated and therefore resistant to digestion with either *Hha* I or *Hpa* II. The transcriptionally inactive κ^- alleles in the other two plasmacytomas (PC 3386 and PC 10916) were also found to be unmethylated at sites within a few kilobases of the C_κ gene, thus resembling the κ^+ and transcriptionally active κ^- alleles in this regard. At more distant sites—e.g., 4 kb upstream of an expressed *V* gene in PC 6684—approximately 20% methylation is observed. Likewise, the *Hpa* II/*Msp* I sites 5.0 and 8.4 kb downstream from the C_κ gene (M_1 and M_2) are methylated to varying extents in the different plasmacytomas.

DISCUSSION

The present findings, as well as those reported previously (12, 13), have clearly demonstrated that the chromatin encompass-

ing the J_κ–C_κ region (herein termed the κ locus) has a distinctive "open" structure in plasmacytoma cells, which results in its greater sensitivity to DNase I digestion compared to chromatin containing nonexpressed genes. Although the chromatin containing a V_κ gene does not have this property when the V_κ gene is in its germline context, it acquires DNase I-sensitive structure when fused to the κ locus. Our studies have further demonstrated that the κ locus is constitutively hypomethylated in plasmacytomas and their hybridoma derivatives. In contrast, germline V_κ genes are in more highly methylated regions of DNA. As with DNase I sensitivity, the V_κ genes become hypomethylated when fused to the κ locus. The fact that these properties are not acquired by closely related adjacent V_κ genes or by the identical allelic V_κ gene in germline context indicates that this is a localized *cis*-acting phenomenon. Hypomethylation was found to be propagated to sites at least 4 kb, but not more than 7 kb, upstream of the transcriptional start site. Thus, the transcriptional competence of a V_κ gene is highly correlated with open chromatin structure and hypomethylation, which in turn appears to be dependent on proximity to the J_κ–C_κ region.

As we have pointed out elsewhere (10), transcription at the κ locus also requires a proper initiation signal, which is normally supplied by the incoming V_κ gene or, in the case of the κ^0 locus, by a fortuitously located pseudo initiation signal. In some plasmacytomas there are κ^- alleles resulting from rearrangements that do not supply proper initiation signals to the κ locus. Although transcriptionally inactive, these κ^- loci are found to maintain a hypomethylated status. In one such plasmacytoma, PC 7183, the unmethylated *Hpa* II site is known to lie within a segment of chromosome 15 that is brought to the κ locus by chromosomal translocation (26). However, RNA transfer blot analyses indicate that no stable transcripts are produced from this translocated segment. Thus, persistent transcriptional activity does not appear to be the cause of the undermethylation of the κ locus. Rather, a hypomethylated state is more likely to be one of the prerequisites of transcriptional competence, as has been inferred from experiments in which transcription and undermethylation are co-induced by treatment with 5-azacytidine (2, 27, 28) or experiments in which expression of viral genes is abolished by methylation of specific *Hpa* II sites (29, 30).

There is reason to believe that the heavy chain (H) locus is also subject to this general type of regulation. Unrearranged (H^0) alleles and incompletely rearranged (H^-) alleles—e.g., *D–J* (diversity–joining) fusions, are transcriptionally active (31–33). This transcription, which is initiated on both sides of the J_H region and continues beyond the C_μ region, occasionally occurs in T-cell derivatives as well as in cells of B-lymphoid lineage. Moreover, the J_H–C_μ locus is usually in an undermethylated state, whereas other heavy chain constant region genes—e.g., $C_\gamma 2b$ or $C_\gamma 1$—remain methylated until fused to the H locus by switch recombination (34, 35).

An important question that remains to be answered is "What creates the open, hypomethylated structure of the κ locus in lymphoid cells?" This condition could be established by the interaction of some tissue-specific factor with elements in the J_κ–C_κ region that influence DNA supercoiling or that maintain a higher order loop structure at this locus. The finding of DNase I-hypersensitive sites in the J_κ–C_κ intron in the vicinity of an evolutionarily conserved A+T-rich region and sequences with homology to a putative simian virus 40-enhancer element (13, 36, 37) lends some credence to this possibility. If such sequence elements contribute to the distinctive structure of the κ locus, then one might consider methylation or hypomethylation as a means of stabilizing the structure in an inactive or active state.

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