Structure of a nuclease-sensitive region inside the immunoglobulin kappa gene: evidence for a role in gene regulation

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ABSTRACT

A discrete chromatin region inside the active immunoglobulin kappa gene is preferentially accessible to cleavage by nucleolytic enzymes. This region comprises 200-250 bp of DNA, and is situated within the large intron of the gene, approximately 600 bp upstream from the constant region coding sequence. The local chromatin structure of this region correlates with tissue-specific kappa gene expression: it is resistant to nucleolytic digestion in the inactive kappa genes of murine brain and liver nuclei, but becomes uniquely sensitive to cleavage by deoxyribonuclease I or by a variety of restriction endonucleases in the chromatin of kappa-producing cells. Nuclease sensitivity at this site occurs in both rearranged and unrearranged kappa alleles, and can be maintained in the absence of ongoing kappa transcription. The nucleotide sequence of the hypersensitive region has been selectively conserved in evolution, and includes both a 7 bp inverted repeat sequence and a short segment homologous to the transcriptional enhancer elements of certain eukaryotic viruses. Molecular events occurring at this locus may play a role in the regulation of kappa gene expression, perhaps by influencing the activity of promoter sequences several kilobases upstream.

INTRODUCTION

The distinctive patterns of gene expression which define individual cell types are reflected in the tissue-specific structure of chromatin (reviewed in ref. 1). Transcriptionally active genes are maintained in a chromatin conformation which is preferentially susceptible to the nucleolytic action of pancreatic deoxyribonuclease I (DNAase). In addition, small (<300 bp) foci of altered chromatin structure, in which the underlying DNA is exquisitely sensitive to DNAase cleavage, have recently been described (2,3). Such hypersensitive sites are distributed throughout the genome, frequently occurring near the 5' or 3' end of an active or potentially active transcription unit. These focal alterations in chromatin structure have been implicated in the control of gene function because, in many instances, DNAase hypersensitivity of such sites correlates with gene activity (4-10), and because the locations of these sites often coincide with DNA sequences believed to have a role in gene regulation (11-13).

Investigations in several laboratories have suggested that chromatin structure may influence the expression of immunoglobulin kappa light chain genes. Each functional kappa gene is the product of a somatic DNA rearrangement event known as V/J joining, in which a single variable region (V_K) coding element, along with its 5' flanking sequences, is fused to one of four joining (J_K) genes located 2.5-3.9 kb upstream of the constant region (C_K) coding sequence (14,15). Transcription of the assembled gene is initiated at the 5' end of the V_K locus. The murine genome includes a large and diverse family of non-allelic V_K genes, each harboring a potential initiation site at its 5' end. Although their promoters are capable of directing a low rate of initiation in cell-free or oocyte transcription systems (16), unrearranged (germline) V_K genes of immunoglobulin-producing cells are maintained in a chromatin state resistant to DNAase digestion (17), and are not transcribed (18). The process of V/J joining imparts DNAase sensitivity to the rearranged V_K locus, and produces an increase of greater than 10,000-fold in the activity of its promoter, as measured in native myeloma chromatin (17,18). Cloned rearranged kappa genes introduced into lymphoid recipient cells can be transcribed (19-21). However, when an entire rearranged kappa transcription unit was cloned from myeloma cells and transfected into non-lymphoid recipient cells, the V_K promoter was found to be inactive, implying that proper rearrangement of the DNA sequence alone is not sufficient to activate the gene (22).

We have recently reported a specific change which occurs in the chromatin structure of the kappa genes during induction of kappa light chain expression in the murine pre-B lymphocytoid line 70Z/3 (23). Cells of this line contain a single rearranged kappa gene, but ordinarily express neither kappa light chain protein nor its corresponding messenger RNA (24-26). On exposure to bacterial lipopolysaccharide (LPS), however, the cells begin to accumulate cytoplasmic kappa-specific mRNA; after 12 hr of optimal LPS treatment, 70-100% of the population expresses surface and cytoplasmic kappa protein. LPS treatment apparently activates transcription of the constitutively-rearranged kappa gene without further DNA rearrangements; the excluded kappa allele retains the germline configuration and is not detectably transcribed (Parslow et al, manuscript in preparation). In untreated 70Z/3 cells, both the rearranged and germline kappa alleles are preferentially susceptible to DNAase digestion as compared to inactive chromatin, but no DNAase hypersensitive sites are observed within several kilobases of either gene. When kappa expression is induced by exposure to LPS, however, a discrete region closely

linked to the C_K coding sequence undergoes a change in chromatin structure which renders it hypersensitive to DNAase (23). Hypersensitivity at this site arises simultaneously on both kappa alleles, but does not occur elsewhere in the vicinity of either gene. In this report, we demonstrate that this region is uniquely accessible to the actions of a variety of nucleolytic enzymes, and present a detailed analysis of the sequence and structure of the inducible hypersensitive site. In addition, we demonstrate that changes in the local chromatin organization of this site correlate with kappa gene expression in a tissue-specific fashion, and propose a role for these chromatin changes in the induction of kappa gene transcription.

METHODS

70Z/3 cells were grown at a density of 0.5–2.0 × 10⁶ cells/ml in suspension culture at 37°C under an atmosphere of 7% CO₂. The culture medium was RPMI-1640 supplemented with 10% fetal calf serum, antibiotics, and 50 μM β-mercaptoethanol with or without the addition of 10 μg/ml LPS from Salmonella typhosa 0901 (Difco). The induction of kappa expression in LPS-treated cultures was confirmed by surface fluorescence immunoassay and by RNA blot hybridization (27).

Nuclei were prepared from washed cells by gentle homogenization in a solution containing 0.25 M sucrose, 10 mM Tris, pH 8.0, 10 mM MgCl₂, and 1% (w/v) Triton X-100, then washed three times in the same solution without detergent. For restriction nuclease digestions, nuclei were suspended at a DNA concentration of 1 mg/ml in ice-cold 0.25 M sucrose, 10 mM Tris, pH 8.0, 30 mM NaCl, 16 mM MgCl₂, 6 mM β-mercaptoethanol, 0.1 mg/ml bovine serum albumin, and 0.1 mM phenylmethylsulfonylfluoride (PMSF). Restriction enzymes (all obtained from New England Biolabs) were added to 0.25 ml aliquots of the nuclear suspensions, and incubated at 37°C for 30 min. Digestions were terminated by the addition of 0.1 volume each of 10% SDS and 2 mg/ml proteinase K. Restriction enzymes were ordinarily employed at a final concentration of 160 units/ml; the use of higher concentrations generally produced significant bulk degradation of chromatin without increasing specific cleavage. The optimal temperature for Bst NI digestion is 67°C; because its activity was reduced three- to five-fold under the conditions used here (37°C), this enzyme was used at a nominal concentration of 460 units/ml. With the exceptions of Dde I and Alu I, the restriction enzymes produced only minimal bulk chromatin degradation under the conditions employed here.

Digestion of nuclei with S1 nuclease was performed as described by Larsen

and Weintraub (28), in a solution containing 0.25 M sucrose, 30 mM sodium acetate, pH 4.6, 50 mM NaCl, 1 mM ZnSO₄, and 0.1 mM PMSF. Procedures for DNAase digestion of nuclei, for purification of digested DNA from nuclei, and for hybridization analysis of DNA have been described previously (23). The C_K probe was prepared by combined Hin cII and Hin fI digestion of cloned kappa complementary DNA. (unpublished) The probe for upstream intronic (IVS) sequences was prepared by combined Hin dIII and Xba I digestion of a cloned embryonic kappa locus, kindly provided by E. E. Max and P. Leder.

RESULTS

Fig. 1A depicts the structures of the two kappa alleles of 70Z/3, along with the hybridization probes used in these studies. The restriction map of the germline locus is identical to that reported previously for mouse embryonic DNA (29). The organization of the rearranged kappa allele of 70Z/3 was determined by analysis of recombinant phage harboring this gene; the complete nucleotide sequence of the variable region and 5' flanking DNA will be presented elsewhere (Parslow et al., manuscript in preparation). V/J joining was found to have occurred at the farthest upstream (J1) joining locus in the rearranged gene. A Bam HI restriction site is located near the middle of the V_K coding sequence, 166 bp upstream from the site of V/J joining. In the present report, we confine our analysis to the region downstream of this Bam HI site, a region which does not include the extreme 5' end of the rearranged transcription unit.

70Z/3 cells were grown for 18-24 hr in the presence of 10 µg/ml LPS, conditions sufficient to induce the expression of kappa transcripts and of surface kappa protein in >80% of the population. Nuclei were isolated from these cells and digested with one of several nucleolytic enzymes. To determine the effects of such digestions on kappa-specific chromatin, the DNA contained in these nuclei was then purified, cleaved to completion with Bam HI (either alone or in combination with other restriction enzymes), subjected to agarose gel electrophoresis, transferred to nitrocellulose membranes, and probed for kappa gene sequences. When analyzed in this fashion, using a probe for C_K sequences, DNA from undigested nuclei revealed two distinct hybridizable restriction fragments, corresponding to the two kappa alleles. (Fig. 1B, lane 1) We have previously demonstrated (23) that mild DNAase digestion of nuclei from LPS-treated cells, followed by Bam HI digestion of the purified DNA, gives rise to a third C_K-bearing species -- a 2.1 kb subfragment produced by preferential DNAase cleavage at a discrete site within

Figure 1. A DNAase Hypersensitive Site in the Kappa Chromatin of LPS-treated 70Z/3 Cells.

(A) Organization of the two chromosomal kappa alleles of 70Z/3. C, constant coding and 3' untranslated regions; J, junctional coding elements; V, the rearranged variable region gene; Bam, Bam HI; R1, Eco RI; X, Xba I; DNAase, the hypersensitive site. By convention, the farthest upstream J element is designated J1. The location of J3, a non-functional pseudogene, is not shown. The restriction maps of the two alleles are identical throughout the regions downstream of J1; only Xba I sites common to both alleles are depicted here. The specificities of the C_K and IVS probes are also illustrated.

(B) DNAase digestion of kappa genes in the nuclei of LPS-treated 70Z/3 cells. Nuclei were incubated for 2 min at 37°C in the presence of pancreatic deoxyribonuclease I (DNAase, Worthington DPFP grade) at concentrations ranging in equal increments from 0 mg/ml (lane 1) to 4 mg/ml (lane 7). DNA purified from these nuclei was digested with a combination of Bam HI and Eco RI, fractionated on a 0.6 % agarose gel, transferred to nitrocellulose, and probed for C_K sequences. K⁰ and K⁺ denote the germline and rearranged kappa alleles, respectively.

the large kappa intron. (Fig. 1B, lanes 2-7) The approximate location of this DNAase hypersensitive site is indicated in Fig. 1A.

A Cluster of Accessible Restriction Sites in Kappa Chromatin

We first sought to determine whether the modified chromatin structure of the DNAase hypersensitive site makes it susceptible to the action of restriction endonucleases. Nuclei from LPS-treated cells were incubated at 37°C for 30 min in the presence of selected restriction enzymes, each having one or more potential cleavage sites within the kappa intron. After

Figure 2. A Discrete Region Within the Kappa Intronic Chromatin of LPS-treated 70Z/3 Cells is Accessible to Restriction Enzyme Digestion.

(A) Preferential restriction enzyme cleavage of the chromosomal kappa genes. Nuclei from LPS-treated 70Z/3 cells were incubated for 30 min at 37°C in the presence or absence of various restriction endonucleases, each at a concentration of 160 units/ml. DNA was then isolated from the nuclei, cleaved to completion with Bam HI, subjected to electrophoresis on a 0.6% agarose gel, and probed for C_{κ} sequences. Each lane contains 10 μ g DNA. Lane 1 contains DNA from unincubated 70Z/3 nuclei; lane 2 is from nuclei incubated in the absence of added enzymes. K^0 and K^+ denote the germline (11.5 kb) and rearranged (5.6 kb) kappa alleles, respectively.

(B) Accessible restriction sites in the vicinity of the kappa genes. The locations of potential cleavage sites are indicated for each of the eight restriction enzymes tested; arrows indicate the sites of preferential cleavage in 70Z/3 nuclei exposed to each enzyme. The enzymes Hae III and Bst NI cleaved avidly, with little non-specific chromatin degradation (solid arrows); we were unable to determine the relative degrees of cutting at the two sites accessible to each of these enzymes. Open arrows indicate the more weakly accessible Hin FI and Dde I sites. All batches of Dde I tested were found to produce extensive non-specific chromatin degradation, which was only partially inhibited by the inclusion of 0.1 mM PMSF in the reaction mixture. Consequently, it was difficult to quantify the extent of preferential Dde I cleavage at the site indicated, and its designation as a weakly accessible site is, in part, arbitrary. No cleavages were observed outside the region shown. Numbering of residues follows Max et al. (29) to facilitate comparison with the sequence of the intron.

purification and secondary cleavage with Bam HI, the DNA was probed for C_{κ} sequences. A representative autoradiogram is shown in Fig. 2A. DNA from unincubated nuclei (lane 1) revealed the intact germline (K^0) and rearranged (K^+) kappa alleles. No apparent degradation of these fragments occurred when nuclei were incubated in the absence of restriction enzymes. (lane 2) Similarly, the addition of certain enzymes, such as Ava II, Hin dIII, or Sau 3AI, produced neither specific cleavages nor bulk chromatin degradation.

Other enzymes, however, were capable of cleaving kappa-specific chromatin. In nuclei treated with either Hae III (lane 6) or Bst NI, the majority of hybridizable material was contained in a single prominent 2.1 kb subfragment, while the intensity of the larger parent fragments was correspondingly reduced. A slightly larger (2.2 kb) C_{κ} -bearing subfragment was generated by exposure to Dde I, although its presence was frequently obscured by non-specific chromatin degradation produced by the enzyme preparation. (lane 5, see also Figs. 3 and 5) In contrast, exposure to Alu I also resulted in extensive non-specific chromatin degradation, but generated no discernible subfragments. The enzyme Hin fI produced only faint traces of specific cutting, giving rise to a 1.9 kb subfragment.

Detailed analysis of these nuclear digests allowed us to map the precise chromatin sites accessible to each restriction enzyme. The results are summarized in Fig. 2B; additional data in support of these conclusions appear in Figs. 3, 4, and 5. Four of the enzymes tested produced specific cleavage near the kappa genes; all of the observed cleavages occurred inside the large intron. Within the limits of resolution of our electrophoretic analysis (approximately ± 60 bp), each accessible site coincided with a recognition sequence for the appropriate enzyme, as predicted from the known intronic sequence. The enzymes Hae III and Bst NI each cleaved selectively at two accessible sites within a region 530-670 bp upstream from the 5' end of the C_{κ} coding sequence (solid arrows), but did not cleave sites elsewhere in the gene. Preferential Dde I cleavage occurred with variable efficiency at an adjacent site 735 bp upstream from the C_{κ} exon. This compact cluster of accessible restriction sites was flanked downstream by a single weakly accessible Hin fI recognition sequence; all other potential restriction sites in the vicinity of the gene were comparatively resistant to nucleolytic attack.

Boundaries of the DNAase Hypersensitive Region

Because the sites of restriction nuclease cleavage in kappa chromatin were precisely known, we used these as markers to identify boundaries of the DNAase hypersensitive region in LPS-treated cells. Samples of DNA from nuclei treated either with restriction enzymes or with DNAase were secondarily cleaved with Bam HI, applied to agarose gels, and transferred to nitrocellulose membranes. Analysis of the genomic digests with a C_{κ} -specific probe revealed that the 3' boundary of the DNAase hypersensitive region approximately coincides with the locations of the accessible Hae III and Bst NI sites, but does not extend downstream to the weaker Hin fI site.

Figure 3. A Variety of Nucleolytic Enzymes All Cleave Preferentially Within a 200–250 bp Segment of the Kappa Intron in 70Z/3 Chromatin.

Nuclei prepared from LPS-treated cells were either incubated with restriction enzymes as described (legend to Fig. 2), or digested with DNAase (0.4 mg/ml) for 2 min (see legend to Fig. 1). DNA purified from these nuclei was secondarily cleaved with Bam HI, then probed for specific gene sequences.

(A) Samples probed for constant region sequences after electrophoresis on a 1.1% agarose gel, with 25 μ g DNA per lane.

(B) Identical samples probed for upstream intronic sequences following 0.6% agarose gel electrophoresis.

Control samples (lanes 1 and 8) were incubated for 30 min at 37° in the absence of added enzymes. Sizes of the germline (K^0) and rearranged (K^+) kappa alleles are 11.5 kb and 5.6 kb, respectively.

(Fig. 3A) When these same DNA preparations were probed for upstream intronic (IVS) sequences, each enzyme gave rise to two distinct subfragments. (Fig. 3B) The sizes of these subfragments (3.4 kb and 9 kb) indicate that accessibility to restriction nuclease digestion, like DNAase hypersensitivity, occurs at approximately the same location on both the rearranged and germline kappa alleles in LPS-treated cells. DNAase hypersensitivity on the rearranged allele was found to extend as far upstream as the accessible Dde I site. Our analysis therefore indicates that a discrete intronic region, 200–250 bp in length, is both hypersensitive to DNAase and accessible to Hae III, Bst NI, and Dde I restriction endonuclease digestion in the nuclei of LPS-treated 70Z/3 cells. All restriction sites within this region are accessible to cleavage, with the exception of a solitary Sau 3AI recognition sequence near

the middle of the hypersensitive region (see below). Even under conditions of very mild DNAase digestion, we have not detected evidence of heterogeneity in the 5' or 3' boundaries of the hypersensitive chromatin, suggesting that the genomic DNA within this region is uniformly hypersensitive to DNAase.

Tissue-Specificity of Nuclease Digestion

We next examined the chromatin organization of the kappa genes of murine brain and liver, tissues in which these genes are not expressed. Because immunoglobulin gene rearrangements do not occur in these cells, Bam HI cleavage of purified DNA from undigested brain nuclei revealed only a single 11.5 kb C κ -bearing fragment. (Fig. 4A, lane 1) Digestion of brain nuclei with various concentrations of DNAase failed to produce discernible subfragments, indicating the absence of DNAase hypersensitive sites in these inactive genes. (lanes 2-5) Weischet et al. (30), have reported a similar lack of DNAase hypersensitive chromatin in the unexpressed kappa genes of mouse liver. Incubation of murine brain (lanes 6-8) or liver (lanes 9 and 10) nuclei with the restriction endonucleases Hae III or Bst NI produced essentially the same result: the vast majority of kappa genes in each cell population proved inaccessible to nucleolytic attack in the chromatin of non-lymphoid cells. (Fig. 4B)

Analysis of nuclei from 70Z/3 cells grown in the absence of LPS, however, revealed that both the germline and rearranged kappa alleles were accessible to cleavage by Bst NI, despite the absence of detectable kappa gene expression in these cells. (Fig. 4C) Under the conditions employed in these studies, the extent of Bst NI cleavage was approximately identical to that seen in nuclei from LPS-treated cells (lanes 11 and 12). In contrast, S1 nuclease, which has been found to cleave certain other DNAase hypersensitive sites (28), had no effect upon the kappa intron in nuclei from either LPS-treated or untreated 70Z/3 cells. (lanes 13 and 14)

The ability of Bst NI to cleave the kappa chromatin of untreated 70Z/3 cells was surprising in view of our earlier observation (23) that no DNAase hypersensitive sites are detectable in these genes prior to activation by LPS. (Fig. 5A) In order to confirm the accessibility of the inactive kappa genes to restriction cleavage, nuclei isolated from 70Z/3 cells grown in the absence of LPS were digested with Bst NI, Dde I, or Hae III. Purified DNA from these nuclei was then digested to completion with a combination of Bam HI and Xba I, subjected to agarose gel electrophoresis, and probed for sequences upstream of the hypersensitive region. DNA from similarly digested nuclei of LPS-treated cells was included for comparison. As illustrated in Fig. 5B, this analysis

Figure 4. Nuclease Sensitivity of Kappa Intronic Chromatin is Tissue-Specific.

(A) Absence of DNAase hypersensitive sites in murine brain chromatin. Nuclei isolated from the brains of BALB/c mice were digested with increasing concentrations of DNAase, ranging in equal increments from 0 to 4.0 mg/ml, as described. (legend to Fig. 1) DNA purified from these nuclei was cleaved with *Bam* HI, electrophoresed on a 0.8% agarose gel (15 μ g DNA/lane), and probed for C_{κ} sequences.

(B) Similar preparations of murine brain (lanes 6-8) or liver (lanes 9 and 10) nuclei were incubated in the presence of either *Hae* III (160 units/ml) or *Bst* NI (nominally 460 units/ml), or without added enzymes (lanes 6 and 9). After secondary cleavage with *Bam* HI, the purified DNA (10 μ g/lane) was analyzed on an 0.8% agarose gel and probed for C_{κ} sequences.

(C) Nuclei prepared from 70Z/3 cells grown in the presence or absence of LPS were digested with either *Bst* NI (nominally 460 units/ml) or S1 nuclease (1080 units/ml) at 37°C for 30 min. Purified DNA was then cleaved with *Bam* HI, fractionated on 0.6% agarose gels (25 μ g DNA/lane), and probed for upstream intronic (IVS) sequences. K^0 and K^+ denote the germline and rearranged kappa alleles, respectively.

failed to reveal any qualitative effect of LPS treatment on the susceptibility of the kappa intron to restriction digestion. All three enzymes were capable of cleaving the kappa chromatin of untreated 70Z/3 cells at the same sites which had been found to be accessible in LPS-treated cells.

Taken together, these findings indicate that preferential accessibility of this small chromatin segment to restriction nuclease digestion can exist in the absence of demonstrable hypersensitivity to DNAase. Although DNAase hypersensitivity correlates well with kappa gene transcription, a cluster of accessible restriction sites can be detected prior to kappa gene activation in cells developmentally committed to the expression of these genes. Data

Figure 5. Effect of LPS Treatment on the Nuclease Sensitivity of Kappa Chromatin in 70Z/3 Cells.

(A) Induction of DNAase hypersensitivity by LPS treatment. Nuclei from LPS-treated or untreated cells were incubated in the presence or absence of DNAase (1 mg/ml) as described. (legend to Fig. 1) DNA isolated from these nuclei was digested with *Bam* HI, analyzed on a 0.6% agarose gel, and probed for C_k sequences. The germ line (K^0) and rearranged (K^+) kappa alleles are indicated.

(B) Accessible restriction sites in the kappa chromatin of LPS-treated and untreated cells. After incubation of nuclei with various restriction enzymes for 30 min at 37°C, purified DNA was cleaved to completion with both *Bam* HI and *Xba* I. Samples containing 20 μ g DNA were analyzed on a 1.1% agarose gel, transferred to nitrocellulose, and probed for upstream intronic (IVS) sequences. K denotes the single parental IVS-bearing fragment derived from both kappa alleles.

presented by McGhee, et al. (31), suggest a similar disparity in nuclease sensitivity at the 5' end of the chicken βA globin gene: in the nuclei of primitive erythrocytes from 5-day embryos, which do not express βA globin, this region can be partially cleaved by high concentrations of the restriction enzyme *Msp* I, but is not hypersensitive to DNAase.

Sequence of the Nuclease-Hypersensitive Region

The nucleotide sequence of this hypersensitive region and adjacent intronic DNA was determined by analysis of the cloned rearranged kappa gene from 70Z/3, and is presented in Fig. 6A. Within the 320 bp region depicted here, the sequence of the rearranged gene differs from that reported for murine embryonic DNA (29) at only three residues. The locations of the accessible *Bst* NI, *Dde* I, and *Hae* III restriction sites are indicated by

This sequence was carefully examined in an attempt to identify features which might account for its distinctive properties in chromatin. Although DNAase is known to cleave preferentially in regions of DNA having a high

proportion of AT base pairs (32), neither the hypersensitive site itself nor its flanking DNA is significantly AT-rich. Conversely, no DNAase hypersensitive sites have been observed in the large AT-rich segment of the kappa intron, which extends 100 to 700 bp upstream of the region shown. The short palindromic sequences associated with sites of DNA recombination for V/J joining do not occur near the hypersensitive region, and no significant homology with the RNA splice junctions of the J or C region coding elements could be detected (29). We were also unable to detect homology between this sequence and the putative initiation site for V_k gene transcription (33).

The nuclease-hypersensitive region thus occurs in a region of the kappa intron which has neither an identified function nor detectable homology to known functional elements in the gene. In comparing the sequences of cloned human and murine kappa genes by heteroduplex analysis, however, Hieter et al. (34) have observed that the sequence of a short (150 bp) segment of intronic DNA has been selectively conserved in evolution, while the remainder of the intron has diverged extensively. Although the precise nature of this conserved region has not yet been determined, its location appears to coincide closely with that of the nuclease-hypersensitive chromatin. This selective evolutionary conservation implies a functional role in kappa gene biology.

DNA Conformation in the Hypersensitive Region

A remarkable feature of the DNA sequence shown in Fig. 6A is the presence of a 7 bp inverted repeat symmetry (CTGGCAG-CTGCCAG) within the hypersensitive chromatin. (arrows) This heptanucleotide sequence is not found, in either orientation, at any other location in the kappa gene. In the hypersensitive region, a distance of 23 bp separates the two halves of the dyad. In theory, base-pairing interactions between the complementary halves of this inverted repeat sequence could form an intrastrand hairpin or stem-loop structure which might, in turn, give rise to the distinctive chromatin organization of the region. (Fig. 6B) Although such a structure might be thermodynamically disfavored in solutions of naked DNA, local torsional forces or interactions with DNA binding proteins could theoretically stabilize this hairpin conformation in the nucleus. Similar regions of intrastrand complementarity have been observed at DNAase hypersensitive sites in both polyoma virus and the chicken β^A globin gene (28,35). It has been suggested that unique secondary structures produced by hairpin formation may determine the local chromatin organization of these sites, perhaps by facilitating the recognition of particular nucleotide sequences by DNA-binding proteins (28).

In the kappa intron, a Sau 3AI restriction site occurs midway between the

two copies of the heptanucleotide repeat. (Fig. 6A) Formation of a hairpin structure by intrastrand base-pairing would disrupt the double-stranded character of this restriction site, thereby inhibiting cleavage by the Sau 3AI enzyme. Significantly, we have been unable to detect cleavage at this site in nuclei from LPS-treated 70Z/3 cells (Figs. 2 and 3), although adjacent sites both upstream and downstream are accessible to cleavage by other restriction enzymes. Even with maximal intrastrand base-pairing, however, such a hairpin structure might be expected to contain single-stranded regions accessible to S1 nuclease digestion; our inability to detect S1 or Sau 3AI cleavage within the hypersensitive region (Fig. 4) may indicate either that Sau 3AI cannot efficiently digest nuclear chromatin, or that some steric feature of the local chromatin structure protects this restriction site from nucleolytic attack.

DISCUSSION

We have presented data which indicate that changes in the chromatin conformation of a small intronic locus correlate with kappa gene expression. In the unexpressed kappa genes of murine brain or liver chromatin, this region is relatively inaccessible to the actions of a variety of nucleolytic enzymes, a property it shares with the V_K , J_K , and C_K genetic elements. Its structure is markedly different, however, in the rearranged, transcriptionally-active kappa gene of LPS-treated 70Z/3 cells, where this small chromatin region becomes uniquely susceptible to cleavage by DNAase and by restriction endonucleases.

Several observations indicate that this nuclease hypersensitivity is not simply a consequence of kappa gene rearrangement or transcription. The intronic locus in the unrearranged kappa allele of LPS-treated 70Z/3 cells is accessible to both DNAase and restriction nuclease digestion, although this allele is not detectably transcribed (Parslow et al., in preparation). Restriction enzymes also cleave preferentially at this site in the chromatin of untreated 70Z/3 cells, suggesting that certain changes in local chromatin structure actually precede transcriptional activation of the gene. No other nuclease hypersensitive regions occur in or near the kappa genes of 70Z/3 cells. In addition, the finding that this same site is hypersensitive to DNAase in several kappa-producing murine plasmacytoma lines (30,36) indicates that such chromatin changes may be a general feature of B lymphocyte differentiation, and are not unique to LPS-responsive cells.

Features of DNA structure which lead to the formation of nuclease hypersensitive chromatin have not yet been identified. In this regard, certain physical properties of the kappa intronic site differ strikingly from

those of a well-characterized hypersensitive region at the 5' end of the chicken β A globin gene (4,31). Each of these regions spans approximately 200 bp of DNA, and each undergoes tissue-specific changes in chromatin structure which correlate with gene activity. Whereas the globin region is highly enriched in G and C nucleotides (70% GC), including a sequence of 18 consecutive deoxyguanosine residues, the kappa site has an unremarkable nucleotide content (47% GC within the region shown in Fig. 5A) and is free of long homopolymeric tracts. Enzymatic digestion studies of the globin hypersensitive region suggest a complex internal structure: accessible cleavage sites for a number of restriction enzymes occur throughout this 200 bp sequence, but DNAase I and DNAase II cleave preferentially near the center, while micrococcal nuclease cleaves farther upstream. The globin region is resistant to nuclease BAL-31, but is reportedly sensitive to cleavage by S1 nuclease (28). In contrast, strongly accessible restriction enzyme sites in the kappa locus appear to coincide closely with the region of DNAase I hypersensitivity; neither micrococcal nuclease (30) nor S1 nuclease cleaves kappa intronic chromatin.

An additional difference between these two sites may have particular relevance to the mechanism of gene regulation. The dinucleotide CpG occurs at 15 locations within the globin hypersensitive region--six times the number expected from its frequency in the genome as a whole (37); it occurs with an even higher frequency in the nuclease-hypersensitive regulatory sequence at the 5' end of the herpesvirus thymidine kinase gene (13). This dinucleotide is the principal site of cytosine methylation in eukaryotes, a covalent modification of DNA structure which has been implicated in the control of gene expression (38,39). Only a single CpG dinucleotide occurs within the kappa intronic site, however, indicating that a high density of such dinucleotide sequences is not essential for the formation of hypersensitive chromatin. Interestingly, this methylatable sequence is conspicuously uncommon in DNA regions surrounding the kappa hypersensitive locus, occurring only twice in the entire 3' half (1.25 kb) of the intron (29). By comparison, the dinucleotide occurs at approximately its mean genomic frequency (14-17 copies per kb) throughout the 5' half of the intron, and in adjacent regions encompassing the J_{κ} and C_{κ} elements.

Evidence from other systems suggests that, while active genes are probably organized in modified nucleosomal units, nuclease hypersensitivity may arise in regions which are not associated with histone proteins (8,31,40). The kappa intronic locus may also represent such a discontinuity

in nucleosomal organization; indeed, the nuclease-resistant region separating this locus from the weakly accessible Hin FI site downstream is 206 bp in length, a distance sufficient to accommodate a normal core histone octamer. From the standpoint of gene regulation, however, it may be particularly significant that DNAase hypersensitivity arises at the binding domains of such regulatory proteins as TFIIIA (which binds the 5S RNA promoter of Xenopus) or the T antigen of SV40 (8,12). Whatever the factors which produce hypersensitivity at the intronic site, they do not appear to discriminate between the germline and rearranged kappa alleles of 70Z/3, suggesting that they are influenced primarily by short-range sequence parameters.

The nuclease hypersensitivity and selective evolutionary conservation of the kappa intronic locus suggest that it may have a function in the control of kappa gene expression. By analogy with findings in other gene systems, tissue-specific changes in the chromatin organization of this region might be required to induce transcriptional competence in V_K promoters linked to the C_K region. The location of the kappa intronic site is enigmatic, however, since it is by no means clear how a localized change in chromatin structure could influence the activity of a promoter located more than 3.5 kb upstream. Precedent for such regulatory action at a distance may be found in the viral enhancer sequences, short cis-acting genetic elements which can substantially increase the activity of eukaryotic promoters situated several kilobases upstream or downstream (41-44). Significantly, Chung et al. (36) have recently observed an 11 bp region of homology between the kappa intron and the 72 bp enhancer element of SV40; as indicated in Fig. 5A (box), this homologous sequence (GGGGACTTCC) falls within the nuclease-hypersensitive region of the intron. Weiher et al. (45) have independently shown that mutations in this segment of the SV40 sequence abolish its activity as an enhancer of viral gene transcription, and have suggested that related sequences may be a general feature of viral enhancer elements.

Nevertheless, it is clear that the mere presence of the kappa intronic locus is not sufficient to activate the V_K promoter in a non-lymphocytic transcription system (22). We favor the view that additional factors, perhaps unique to cells of the B lymphocyte lineage, are required for efficient kappa transcription. One such factor might be a sequence-specific protein or oligonucleotide which, recognizing a distinctive feature of the intronic regulatory element, interacts with this locus in a manner which enhances transcription from the nearest V_K promoter in cis. The association of such a regulatory element with the C_K region would offer a flexible and economical mechanism

through which to activate transcription of a single rearranged V_K element, while preventing concurrent activation of the remaining elements in this multigene family.

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