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DNase I-Hypersensitive Sites and Transcription Factor-Binding Motifs within the Mouse Eβ Meiotic Recombination Hot Spot

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The second intron of the Eβ gene in the mouse major histocompatibility complex is the site of a meiotic recombination hot spot. We detected two DNase I-hypersensitive sites in this intron in meiotic cells isolated from mouse testes. One site appears to be constitutive and is found in other tissues regardless of whether or not they express the Eβ gene. Near this hypersensitive site are potential binding motifs for H2TF1/KBF1, NFκB, and octamer transcription factors. Gel retardation studies with mouse lymphoma cell nuclear extracts confirmed that each of these motifs is capable of binding protein. The binding of transcription factors may contribute to the enhancement of recombination potential by altering chromatin structure and increasing the accessibility of the DNA to the recombination machinery.

The frequency of meiotic and mitotic recombination is not uniform throughout a chromosome. Eucaryotic recombination hot spots have been well characterized in a number of species including mice (4, 8, 23–25, 40, 49, 50, 58, 59) and fungi (reviewed in reference 37). However, only limited information exists on the molecular mechanisms by which hot spots enhance homologous recombination. Intrachromosomal somatic recombination mediated by variable-diversity-joining region [V(D)J] recombinase leads to immunoglobulin and T-cell receptor gene rearrangements. Plasmid constructs have been used to show that VDJ recombination events are stimulated by transcription. This observation led to the idea that enhanced DNA accessibility potentiates genetic exchange (5, 6). Repeated sequences capable of forming Z DNA and a human minisatellite sequence have been found to enhance recombination in yeast and mammalian cell culture systems, but the molecular mechanism for the effect is unknown (7, 9, 51, 57, 61). Studies on mitotic recombination indicate that genetic exchange in yeast cells, can be stimulated by transcription (55, 60), but recent studies (44) on a hot spot active in yeast meiosis (36, 54) show that transcription is not necessary for hot-spot activity.

Unfortunately, experimental systems that simulate the conditions of meiosis in higher eucaryotes are unavailable, and therefore the molecular basis of meiotic-recombination hot spots in these organisms is obscure. Stimulated by the suggestion that recombination enhancement might result from increased accessibility of DNA sequences to the cell's recombination machinery, we decided to determine the state of DNA accessibility in one of the best-defined mammalian meiotic-recombination hot spots (4, 8, 23, 40, 49). This hot spot is found in the second intron of the class II Eβ gene in the mouse major histocompatibility complex (MHC). Although mouse class II genes are spread out over more than 300 kb, recombination in most crosses between inbred strains is limited to an approximately 3-kb region in the second Eβ intron. In our studies we analyzed the chromatin conformation in this gene segment in somatic cells and in purified meiotic pachytene cells isolated from mouse testes.

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MATERIALS AND METHODS

Animals and cell lines. The standard F1 hybrid B6AF$_1$/J (C57BL/6J female × A/J male) strain was obtained from The Jackson Laboratory. The 2PK-3 lymphoma (ATCC TIB 203) and LTK(−) (gift from P. Jones) cell lines were propagated in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 2 mM glutamine, 100 U of penicillin per ml, 100 μg of streptomycin per ml, and 0.05 mM 2-mercaptoethanol (2PK-3 only).

Isolation of pachytene cells. Pachytene cells were isolated from cells from fresh mouse testes (age, 8 to 10 weeks) by velocity sedimentation (11). Fractions containing at least 90% pachytene cells were confirmed by Nomarski differential interference microscopy. On average, a preparation using 10 testes produced 5×10^8 cells, from which 5×10^7 pachytene cells could be purified.

DNase I digestion experiments. Nuclei from 2PK-3, LTK(−), pachytene, and fresh liver cells were isolated and treated (3 min, 23°C) with DNase I by the method of Burch and Weintraub (10). After *Hin*dIII or *Nhe*I digestion, electrophoresis, and Southern blotting, the isolated DNA was hybridized to probe 3 (gift from J. Kobori and L. Hood) or probe 4 (Fig. 1A).

DNA methylation experiments. DNA from 2PK-3 lymphocytes was digested with *Hin*dIII plus *Msp*I, *Hpa*II, or no additional enzyme; electrophoresed; Southern blotted; and hybridized to probe 3.

Gel retardation. Nuclear extract preparation (modified by D. Sakai) was done by the method of Dignam et al. (12). Protein-binding reaction mixtures (14, 16) contained 2 mM $MgCl_2$, 60 mM NaCl, 10 mM Tris HCl (pH 8.0), 10% glycerol, 2 mM dithiothreitol, 100 μg of bovine serum albumin per ml, 0.1 mM EDTA (pH 8.0), and 1 ng of uniformly labeled (by polymerase chain reaction) 276-mer (Fig. 1C) in each 20 μl of reaction mixture. After incubation at 23°C for 30 min, the reaction mixtures were loaded onto a 5% polyacrylamide gel, and electrophoresis was carried out in 0.5× TBE (0.045 M Tris borate, 0.001 M EDTA, pH 8.0) at 4°C for 2 h at 10 V/cm.

Gel retardation was also performed on the following double-stranded Eβ intron oligonucleotides (only one of the duplex strands is given, and the presumptive binding motifs are shown in capital letters): H2TF1/NFkB 30-mer, 5′-taattttaatGGGATTCCCCatcccttatt-3′ (this sequence also

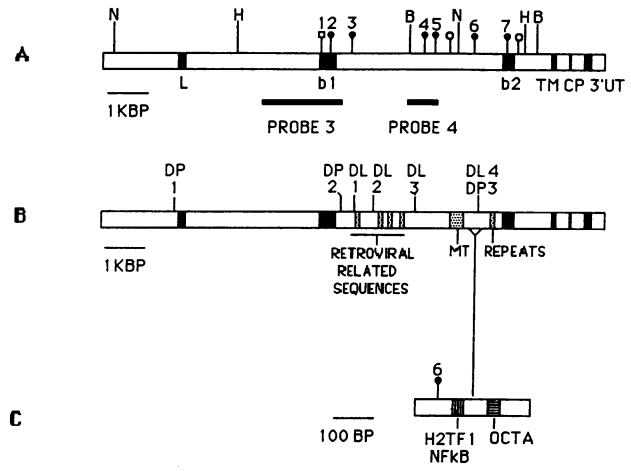

FIG. 1. Mouse Eβ gene. (A) Partial restriction map (23, 41, 63, 64; unpublished data) showing *Hin*dIII (H) and *Bgl*II (B) sites in the *b*, *d*, and *k* haplotypes and the *Nhe*I (N) site in the *b* and *k* haplotypes. *Msp*I-*Hpa*II sites are common to the *b*, *d*, and *k* haplotypes (filled circles 2 through 6), found in *b* and *k* haplotypes (unnumbered open circles), or unique to the *d* haplotype (open square 1). Filled areas indicate the exons. L, Leader; b1 and b2, beta 1 and 2 domains; TM, transmembrane; CP, cytoplasmic; 3′ UT, 3′ untranslated. (B) Locations of DNase I-hypersensitive sites for 2PK-3 lymphoma nuclei (DL1 to DL4) and *b*-*k* pachytene nuclei (DP1 to DP3). Features of the DNA sequence in the intron are noted and explained in the text. (C) The 276-bp polymerase chain reaction product and potential protein-binding sites.

contains a TAATGARAT motif [underlined] with one mismatch [3]); and octamerlike 30-mer, 5′-gcacacacaaaATGA AAATggcctgacttc-3′. Labeling was by polynucleotide kinase. The reaction mix was identical to that used for the 276-mer except that 0.1% Nonidet P-40 was added and each sample contained 3 μg of poly(dI-dC) · poly(dI-dC) (except the control samples without nuclear extract). Incubation was at 23°C for 10 min, and electrophoresis was for 1.5 h in a 6% polyacrylamide gel containing 0.5% Nonidet P-40.

RESULTS

We first used DNase I hypersensitivity to examine the accessibility of the Eβ second intron in somatic cells. The B-cell lymphoma line 2PK-3 expresses the $E\beta^d$ gene (26). Using an assay based on polymerase chain reaction amplification of cDNA, we confirmed the presence of steady-state levels of Eβ mRNA (data not shown). A map of the Eβ gene is shown in Fig. 1A. To study chromatin structure in the region of the recombination hot spot, isolated nuclei from 2PK-3 cells were treated with DNase I; DNA was purified, digested with *Hin*dIII, electrophoresed, blotted, and hybridized to probe 3 (Fig. 1A). When DNA prepared from untreated nuclei of 2PK-3 cells was digested with *Hin*dIII, a major 7.0-kb restriction fragment was observed with probe 3 (Fig. 2, lane 1). When nuclei were exposed to increasing concentrations of DNase I, the 7.0-kb fragment was digested and a prominent band of 6.0 kb and a band of 3.1 kb could be seen. Two minor bands at 4.2 and 2.8 kb could also be detected on the original autoradiographs. These DNase I-dependent fragments allowed the mapping of DNase I-hypersensitive sites DL1 through DL4 (Fig. 1B), all of which fall within the meiotic-recombination hot-spot region defined previously (8, 23, 49). Two additional lymphoma cell lines

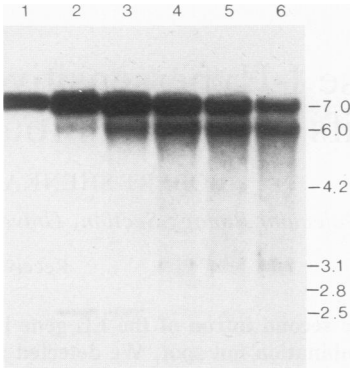

FIG. 2. DNase I-hypersensitive sites in the second intron of the $E\beta^d$ gene of 2PK-3 lymphoma cells. Lane 1, DNA from untreated nuclei; lanes 2 through 6, nuclei treated with 0.5, 1, 2, 3, or 4 U, respectively, of DNase I per ml. The *Hin*dIII-digested DNA was hybridized with probe 3.

(A20 and X16C8.5), which have been reported to express Eβ (21), also exhibited site DL4 (data not shown). Direct DNase I treatment of purified DNA provided no evidence for a region of preferential enzyme digestion within this *Hin*dIII fragment (data not shown). The 2.5-kb band which can be seen even without DNase I treatment is due to cross hybridization with the $A\beta^d$ gene (31) and is discussed further in the legend to Fig. 4.

We also examined hypersensitive sites in the nuclei of mouse somatic cells, which, as documented by others (22), are not known to express class II genes. Figure 3 shows the presence of site DL4 in liver nuclei from F_1 $H2^k/H2^b$ mice. Although the liver Kupffer cells are known to express class II genes, they constitute only a few percent of liver cells and cannot account for the intensity of the 6.2-kb band. We also observed DL4 in the nuclei from LTK(−) fibroblasts (Fig. 4) and brain cells (data not shown). Thus, the DL4 site does not

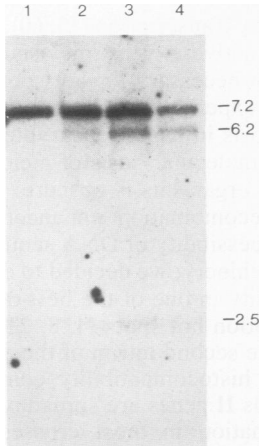

FIG. 3. DNase I-hypersensitive sites in the second intron of the $E\beta^{b/k}$ genes in A/J × C57BL/6J mouse liver cells. Lane 1, DNA from untreated nuclei kept on ice; lane 2, DNA from untreated nuclei kept at 23°C; lanes 3 and 4, nuclei treated with 0.5 or 1 U, respectively, of DNase I per ml. The *Hin*dIII-digested DNA was hybridized with probe 3. The liver *Hin*dIII fragment and the fragment representing DNase I cleavage at D4 are slightly larger than the comparable fragments for 2PK-3 because of differences in the length of the gene in different Eβ haplotypes.

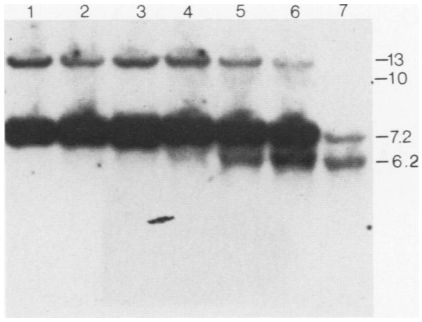

FIG. 4. DNase I-hypersensitive sites in the second intron of the $E\beta^k$ gene of LTK(−) fibroblasts. Lane 1, DNA from untreated nuclei; lanes 2 through 7, nuclei treated with 1, 2, 5, 10, 20, or 50 U, respectively, of DNase I per ml. The *Hin*dIII-digested DNA was hybridized with probe 3. The 13-kb fragment and a 10-kb DNase I-dependent digestion product (seen more clearly on the original autoradiograph) are due to cross hybridization between probe 3 and the related class II $A\beta^k$ gene (47). Aβ cross hybridization with probe 3 can also be seen in mice of the $H2^d$ haplotype which have a cross-hybridizing sequence of 2.5 kb (Fig. 2). In the $H2^b$ haplotype, the $A\beta^b$ *Hin*dIII fragment band is exactly the same size as the $E\beta^b$ band (27).

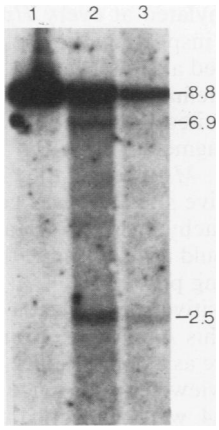

FIG. 6. DNase-hypersensitive sites in the $E\beta^{b/k}$ genes in A/J × C57BL/6J mouse pachytene cells. Lane 1, DNA from untreated nuclei; lanes 2 and 3, nuclei treated with 0.5 or 1 U, respectively, of DNase I per ml. The *Nhe*I-digested DNA was hybridized with probe 4.

appear to be restricted to cells expressing class II genes and therefore may be constitutive.

We next examined nuclei from pachytene cells undergoing meiosis which were isolated from the testes of F_1 hybrid mice ($H2^k/H2^b$). When probe 3 and *Hin*dIII digestion were used, pachytene cells were found to have two principal DNase I-hypersensitive sites (Fig. 5), DP2 and DP3 (Fig. 1B), within the second intron. The position of the relatively weak site DP3 appears to be the same as that of DL4. The intenser DP2 is very close to the 5′ end of the intron, in a region in which we did not observe a hypersensitive site in the B-cell lymphoma or LTK(−) cell lines ($H2^d$ and $H2^k$, respectively), although a weak site was observed in liver cells of F_1 $H2^k/H2^b$ mice (Fig. 3). The weak hybridization seen at 13 and 10 kb is due to cross hybridization with $A\beta^k$ (discussed in the legend to Fig. 4).

To confirm the Eβ origins and positions of DP2 and DP3 in pachytene cells, we carried out confirmatory experiments using a probe (probe 4, Fig. 1A) that does not cross hybridize to the Aβ gene. The sizes of the *Nhe*I (Fig. 6) or *Bgl*II (data not shown) fragments generated by DNase digestion confirmed the localization of DP2 and DP3 within the second intron of Eβ. In addition, because the *Nhe*I restriction fragments include sequences upstream of the *Hin*dIII site, another hypersensitive site (DP1) was found near the leader sequence (Fig. 1B).

DNase-hypersensitive sites are often accompanied by hypomethylation in surrounding areas (reviewed in reference 17). DNA sequence data reveals the presence of seven *Msp*I-*Hpa*II sites within the 7-kb *Hin*dIII fragment encompassing the hot spot in the $H2^d$ haplotype (Fig. 1A; 23, 41; unpublished data). Lane 1 of Fig. 7 shows that digestion by *Hin*dIII alone gave the expected band. *Hin*dIII-*Hpa*II double digestion showed that only a small fraction of the *Hin*dIII

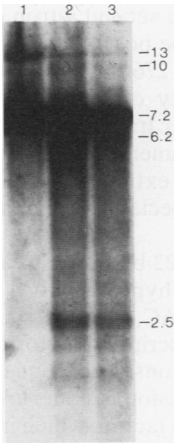

FIG. 5. DNase I-hypersensitive sites in the second intron of the $E\beta^{b/k}$ genes in pachytene cells from A/J ($H2^b$) × C56BL/6J ($H2^k$) mice. Lane 1, DNA from untreated nuclei; lanes 2 and 3, nuclei treated with 0.5 or 1 U, respectively, of DNase I per ml. The *Hin*dIII-digested DNA was hybridized with probe 3.

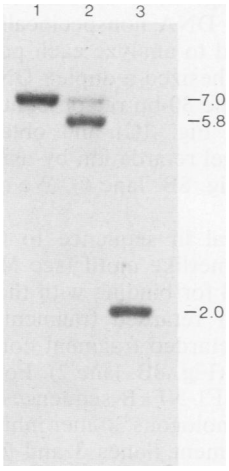

FIG. 7. Methylation in the second intron of the $E\beta^d$ gene in 2PK-3 cells. DNA was digested with *Hin*dIII (lane 1) plus *Hpa*II (lane 2) or *Msp*I (lane 3). Hybridization was with probe 3. The observed sizes for the *Hin*dIII-*Msp*I fragments are 2.0 kb (shown) and 0.4 kb (seen in longer exposures). The expected 94-bp fragment (41) ran off the gel.

fragments were methylated at every *Hpa*II site. As can be readily deduced by inspecting Fig. 7, most *Hin*dIII fragments were methylated at sites 1 to 5 but not at site 6, which is within the region defined by hypersensitive site DL4.

Eight *Msp*I-*Hpa*II sites are present within the corresponding 7.2-kb *Hin*dIII fragments of the $H2^b$ and $H2^k$ haplotypes (Fig. 1A; 23, 63, 64). *Msp*I-*Hpa*II site 2, which is close to DNase I-hypersensitive site DP2, was completely unmethylated in DNA from pachytene cells (data not shown), and as a consequence we could not determine the methylation state of sites 3 to 8 by using probe 3.

DNase I-hypersensitive sites reflect a more-open chromatin conformation. This can result from the disruption of nucleosomal structure as a consequence of a nearby protein-DNA interaction (reviewed in reference 17). The sequences adjacent to DP3-DL4 were examined for known protein-binding sites. We discovered the sequence GGGATTCCCC, which differs by a single base pair deletion from the sequence GGGGATTCCCC. This latter motif lies upstream of the transcription start site of the class I $H\text{-}2K^b$ gene, acts in positive transcriptional regulation, and is bound by the ubiquitous transcription factor(s) H2TF1/KBF1 (1, 2, 18, 32). The GGGATTCCCC sequence is also recognized by the NFκB transcription factor, which is involved in gene expression both in B and non-B cells (reviewed in reference 28). About 73 bp downstream of the H2TF1/KBF1-NFκB site, we noticed a sequence similar to the motif first found to bind octamer transcription factor (ATGCAAAT; 20, 38). The Eβ octamerlike sequence (ATGAAAAT) was identical to a sequence found in the human immunoglobulin H enhancer region. This immunoglobulin H sequence binds mouse myeloma and L-cell nuclear factors (30), although it competes weakly, if at all, with an authentic octamer sequence (48).

We tested a 276-bp fragment containing the two potential protein-binding sites to determine if in fact the sequences we identified were capable of binding protein (Fig. 1C). The fragment was incubated with 2PK-3 lymphoma cell nuclear extract, which would be expected to have high concentrations of the H2TF1/NFκB and octamer factors. Binding was assessed by gel retardation. At least four distinct retarded species could be identified (Fig. 8A, lane 2). As expected, the highest concentration of poly(dI-dC) · poly(dI-dC) weakened specific binding, since specific binding proteins are also known to bind DNA nonspecifically. To simplify our analysis, we decided to analyze each potential binding site separately. We synthesized a duplex DNA fragment identical in sequence to the 30-bp region containing the GGGAT TCCCC motif (23; Fig. 1C) and obtained evidence for protein binding by gel retardation by using nuclear extracts from 2PK-3 cells (Fig. 8B, lane 6). We observed one major retarded fragment.

A 30-mer identical in sequence to the intronic region containing the octamerlike motif (see Materials and Methods) was also tested for binding with the same extract. We observed one major retarded fragment which differed in mobility from the retarded fragment containing the GGGA TTCCCC sequence (Fig. 8B, lane 2). For both the octamerlike and H2TF1/KBF1-NFκB sequences, a 100-fold excess of the unlabeled homologous 30-mer inhibited binding of the major retarded fragment (lanes 3 and 7), while a 100-fold excess of unlabeled heterologous oligonucleotide weakened but did not eliminate binding (lanes 4 and 8). We noticed that the H2TF1/NFκB binding site contains a partially overlapping sequence (see Materials and Methods) which differs by a single base from the TAATGARAT motif which binds purified 100-kDa octamer-binding protein (3). This could explain the weak inhibitory effect of the unlabeled H2TF1/NFκB 30-mer on binding of factors to the labeled octamerlike 30-mer. We conclude that nuclear factors present in mouse lymphoma cells are capable of specifically binding these two motifs in the second intron of the Eβ gene. These data do not exclude the possibility that other novel proteins may bind within the 276-bp segment, which may in fact explain the complexity of the gel retardation pattern observed when this larger fragment is used. In fact, when cold H2TF1 30-mer or octamerlike 30-mer were used as competitors for the binding of extract to the 276-bp fragment, two of the four retarded species were not inhibited (data not shown).

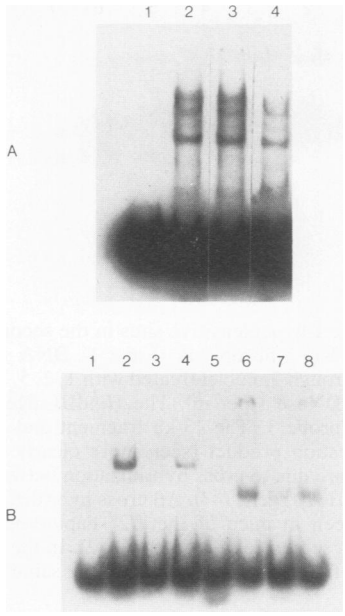

FIG. 8. (A) Gel retardation of the 276-bp fragment (Fig. 1C) by nuclear extracts from 2PK-3 cells. Lane 1, No protein; lanes 2, 3, and 4, protein (1 μg/20 ul) and 1.1, 1.3, and 3.0 μg of poly(dI-dC) · poly(dI-dC), respectively. (B) Gel retardation of 30-bp oligonucleotides (Fig. 1C and Materials and Methods) containing the H2TF1/NFκB or octamerlike sequences by nuclear factors from 2PK-3 cells. Lanes 1 to 4, Retardation of labeled octamerlike 30-mer. Lane 1, No protein; lane 2, 3 μg of protein; lane 3, same as lane 2 but with the addition of 100-fold excess of cold octamerlike 30-mer; lane 4, same as lane 2 but with the addition of 100-fold excess of cold H2TF1/NFκB-like 30-mer. Lanes 5 to 8, Retardation of labeled H2TF1/NFκB-like oligomer. Lane 5, No protein; lane 6, 3 μg of protein; lane 7, same as lane 6 but with the addition of 100-fold excess of cold H2TF1/NFκB-like 30-mer; lane 8, same as lane 6 but with the addition of 100-fold excess of cold octamerlike 30-mer.

We also noted that 22 bp downstream of the second exon (β1) and adjacent to hypersensitive site DP2 was the sequence GGGGCGGAAC, which contains a GC box characteristic of an Sp1 transcription factor-binding site and which conforms to the Sp1 consensus sequence (34).

Class II gene expression is under *trans*-regulatory control by a number of protein factors which interact with sequences immediately upstream of the origin of transcription (reviewed in reference 52). The role of downstream elements in the expression of MHC genes, however, is not understood (15, 29, 39, 53, 62), so any possible significance of our findings to Eβ transcriptional regulation is unknown.

DISCUSSION

We have characterized the DNA accessibility of a mammalian recombination hot-spot region in the mouse testis cell population that is actually undergoing genetic recombination. We find two DNase I hypersensitive sites in the Eβ intron of pachytene cells. One, DP3-DL4, is adjacent to potential binding sites for H2TF1/KBF1, NFκB, and octamer transcription factors. This DNase I-hypersensitive site is found in somatic cells expressing and not expressing class II genes, suggesting that the chromatin organization of this region is constitutive. The H2TF1/KBF1, NFκB, and octamer family proteins appear to have a wide cellular distribution (1, 2, 18, 20, 28, 32, 38, 42, 43), and octamer-binding proteins have been found in male and female primordial germ cells, sperm, and unfertilized oocytes (42, 43). One or more of these transcription factors may be bound to the Eβ intron in meiotic cells, but this possibility needs to be demonstrated directly.

The recombination potential of a chromosomal region may be determined by the interplay among many different factors. In the case of the mouse class II MHC genes, definitive evidence for gene expression in germ cells is lacking (22). Our finding of DNase I-hypersensitive sites, however, raises the possibility that enhanced accessibility of the DNA to the recombination machinery might be facilitated by nucleosomal loss following nearby DNA-protein interactions. Transcription factors present in pachytene cells might be one source of these proteins.

However, the presence of DNase I-hypersensitive sites cannot be the only factor contributing to the activity of the Eβ hot spot. Thus, we detected two hypersensitive sites in regions exhibiting no known genetic recombination activity in inbred mice: DP1 situated 4 kb upstream of the second intron and a site in the Aβ gene (Fig. 6) located 50 kb away.

Another factor that could influence recombination potential is the presence of specific DNA sequences with a high affinity for enzymes involved in recombination. The Eβ intron contains tandemly repeated DNA sequence motifs (consensus, AGGC; 23) having similarity to chi, which increases recombination through its interaction with the *Escherichia coli* enzyme RecBCD (46), and to a core human minisatellite sequence which can undergo addition and deletion events at a high rate (19). Theoretical calculations (33) have shown that these Eβ repeats are also capable of taking on a Z-DNA conformation. The presence of Z-DNA tracts has been experimentally shown to enhance meiotic recombination in yeast chromosomes (57) and plasmids introduced into mammalian somatic cells (9, 51, 61). It is of interest to note that nucleosome formation is inhibited on Z DNA (35).

Still other DNA sequences have been proposed to play a role in Eβ recombination enhancement (45, 64). The Eβ intron also contains (Fig. 1B) retroviral remnants of the long terminal repeat and *gag* and *pol* genes (64), and it is of interest to note that plasmids containing long terminal repeat elements undergo more frequent in vitro recombination than control sequences in mouse testis extracts (13).

Features of the Eβ hot spot region have been compared (45) with features of a second MHC class II mouse recombination hot spot (Aβ3-Aβ2). The Aβ3-Aβ2 hot spot differs from Eβ in that the former enhances recombination only in crosses involving wild-type mouse chromosomes (50, 58, 59), while the latter is detected only in crosses between inbred strains. This reflects a strain or haplotype-specific nature of recombination enhancement. In both hot spots, a member of a mouse-transcribed repeated-sequence family (MT) is found about 1 kb away from an array of tandem 4-bp repeats. The sequence of the Aβ2-Aβ3 tetramer repeat unit (58) differs from that found at Eβ. We have examined the published DNA sequence of the Aβ2-Aβ3 hot-spot region (58) for protein-binding motifs. About 600 bp from the tetrameric repeats and near a long terminal repeat remnant (58), we observed GGGACTCTCC, which matches the NFκB consensus sequence (28) perfectly. At 1,600 bp upstream of the repeats and within 125 bp of each other, we found an Sp1 site and a perfect octamer motif.

While common organizational features of recombination hot spots are of some interest, they probably cannot by themselves explain recombination enhancement (45). For example, at the Aβ2-Aβ3 hot spot, there are no significant differences in DNA sequence between the wild-type mouse chromosomes which do and the inbred mouse chromosomes which do not exhibit recombination enhancement. Shiroishi et al. (45) propose that the overall structural organization of the sequence motifs within the hot spot may determine the site specificity but that enhancers of recombination must exist outside the presently defined Aβ2-Aβ3 hot-spot region in order to explain the frequency differences which exist among mouse strains. Our data suggest another alternative. If protein-binding motifs such as transcription factor-binding sites do in fact contribute to chromatin accessibility in hot-spot regions, then strain differences in the concentration of these factors in meiotic cells might contribute to the haplotype-specific nature of recombination enhancement.

Transcription factor binding could enhance accessibility of the DNA to the cells' recombination machinery and provide one factor contributing to recombination hot-spot activity. It is of interest to note that on the basis of a comparison of genetic maps in eucaryotic species with large and small genomes, Thuriaux (56) proposed that there might be a tendency for recombination to occur preferentially in structural-gene regions.

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