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Genetic control of sex-dependent meiotic recombination in the major histocompatibility complex of the mouse

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Meiotic recombination within the proximal region of the major histocompatibility complex (MHC) of the mouse is not random but occurs in clusters at certain restricted sites, so-called recombinational hotspots. The *wm7* haplotype of the MHC, derived from the wild mouse, enhances recombination specifically during female meiosis within a fragment of 1.3 kb of DNA located between the $A\beta_3$ and $A\beta_2$ genes in genetic crosses with laboratory haplotypes. Previous studies revealed no significant strain differences in nucleotide sequences around the hotspot, irrespective of the ability of the strain to enhance the recombination. It appeared that a distant genetic element might, therefore, control the rate of recombination. In the present study, original recombinants whose breakpoints were defined by direct sequencing of PCR-amplified DNAs were tested for the rate of secondary recombination in the crosses with laboratory strains in order to determine the location of such a genetic element. The results clearly demonstrated that the chromosomal segment proximal to the hotspot is essential for enhancement of recombination. Moreover, the male recombination is suppressed by a segment distal to the hotspot.

Key words: hotspot/meiosis/MHC/recombination

Introduction

Marked sexual differences in rates of meiotic recombination are observed in the human and the mouse. The recombinational map of human chromosomes clearly indicates that average map distance in autosomes is roughly 90% longer in females than in males. Higher rates of recombination during female meiosis are, however, not uniform for the entire length of chromosomes. Map distances in certain parts of some chromosomes appear to be longer in males than in females (Donis-Keller *et al.*, 1987). Similar heterogeneity in the recombinational map with respect to sex has been observed in mouse chromosomes (Davisson and Roderick, 1981). Recent genetic studies of mouse chromosome 16 showed significant sexual differences in recombination frequencies. The differences were also affected by the mouse strains used in the genetic crosses (Reeves *et al.*, 1990). Obviously, such non-uniform sexual differences cannot be explained solely by a generalized increase in *trans*-acting recombinational machinery during female or male meiosis. It may require involvement of a *cis*-acting factor. One attractive interpretation is that there exist sex-dependent recombinational hotspots at which recombination takes place preferentially, either during female or male meiosis. Non-uniform distribution of such hotspots in the genome may result in a mosaic pattern on chromosomes with respect to the sex-related differences in recombination.

The major histocompatibility complex (MHC) is a multigene complex that is present in all vertebrates. The products of the MHC regulate a variety of cellular interactions leading to immune responses. In the mouse, molecular organization of the MHC has been well characterized (for review see Steinmetz, 1986). Recent studies of meiotic recombination in the proximal region of the mouse MHC indicated the presence of at least four independent recombinational hotspots located at different positions. Their locations depend on the allelic form of the MHC haplotypes in genetic crosses (Steinmetz *et al.*, 1982, 1986, 1987; Begovich and Jones, 1985; Saha *et al.*, 1986; Lafuse *et al.*, 1986; Lafuse and David, 1986; Kobori *et al.*, 1986; Uematsu *et al.*, 1986; Zimmerer *et al.*, 1987; Shiroishi *et al.*, 1990). Two MHC haplotypes, *CAS3* and *wm7*, derived from Asian wild mice, show an extremely high frequency of recombination, which are $1 \times 10^2 - 10^3$-fold higher than the rate expected from the physical distance, at the hotspots located between the $A\beta_3$ and $A\beta_2$ genes in genetic crosses with laboratory haplotypes. (Fischer Lindahl *et al.*, 1983, 1986; Steinmetz *et al.*, 1986; Uematsu *et al.*, 1986; Shiroishi *et al.*, 1982, 1990).

A unique feature of the recombination that involves the *wm7* haplotype is sex-dependency. It is only observed in female meiosis and not in male meiosis (Shiroishi *et al.*, 1987). Therefore, it seems to be a candidate for the sex-dependent hotspot discussed above. Comparison of the molecular organization of this hotspot and that of the previously characterized hotspot identified in the $E\beta$ gene revealed that both hotspots have similar configurations consisting of two molecular elements: the consensus sequence of the mouse middle-repetitive MT-family (Heinlein *et al.*, 1986) and the TCTG or CCTG tetramer repeated in tandem several times, separated by about 1 kb of DNA (Shiroishi *et al.*, 1990). Previous genetic studies demonstrated that there is an apparent strain difference in frequency of the recombination at the hotspots. The *wm7* and *CAS3* haplotypes showed high frequencies of recombination in the genetic crosses with different laboratory haplotypes. By contrast, recombination at this hotspot has never been observed in any cross between laboratory haplotypes. Thus, these two wild-derived haplotypes seem to act in a dominant fashion over the laboratory haplotypes in the enhancement of the recombination. In spite of this apparent strain difference, there are no significant differences in the sequences around the hotspot between these wild-derived haplotypes and the laboratory haplotypes. These

features of the hotspot strongly suggest the presence of an element at some distance from the hotspot that controls the rate of recombination. Also the element is active only in certain haplotypes, such as *wm7* and *CAS3*.

In the present study, we first defined the precise breakpoints of 12 independent recombinants from crosses between *wm7* and laboratory haplotypes. Secondly, in order to make a physical assignment of the genetic factor responsible for the strain difference, we used a mating experiment to test the frequency of secondary recombination in crosses between *wm7*-derived primary recombinants and laboratory mice. The results showed that recombinants that have a laboratory derived chromosomal segment in the region proximal to the hotspot were associated with complete absence of recombination at the hotspot. Inversely, the reciprocal recombinants that retained the *wm7*-derived chromosome in the same region still exhibited high level of recombination. Moreover, recombination was observed not only in female meiosis but also in male meiosis. Based on these results, we report here a genetic control of sex-dependent meiotic recombination in the proximal region of the mouse MHC.

Results

Fine mapping of the breakpoints of primary recombinants by direct sequencing of PCR-amplified DNAs

To define the recombinational breakpoints, we amplified a fragment of 1.7 kb of DNA that contained the $A\beta_3/A\beta_2$ hotspot, by means of the polymerase chain reaction (PCR), from 12 independent recombinants whose breakpoints were known to be confined within this hotspot. The recombinants used are listed in Table I. Subsequently, the amplified DNAs were directly sequenced without cloning procedure. Direct comparison of the nucleotide sequences of each recombinant, with those of the parental *wm7* and laboratory haplotypes allowed us to assign the locations of the breakpoints. The results are summarized in Figure 1. None of the 12 recombinants showed a single base deletion or any insertions in the sequenced fragments that covered the hotspot (data not shown). In the genetic cross between the *wm7* and laboratory *b* haplotypes, breakpoints of all four recombinants examined were confined within a segment of a 337 bp of DNA, defined by two polymorphic nucleotides at positions 837 and 1174. In the crosses with laboratory *k* haplotypes, with the exception of B10.A(R201), the breakpoints of seven recombinants were mapped within the segments located between positions 1079 and 1649, while B10.A(R201) had a breakpoint within a segment defined by positions 395 and 1079.

Rate of recombination in the crosses between primary recombinants and laboratory strains

Five recombinant strains whose recombinational breakpoints were precisely determined in the present study, as described above, were tested to see whether they are able to enhance meiotic recombination in the *H-2K-Aβ* interval, which includes the $A\beta_3/A\beta_2$ hotspot. In the mating experiments, B10.A(R206), B10.A(R209), B10.BR(R228) and B10.A(R201) mice were crossed with C57BL/10J (B10) strain. B10(R231) mice were crossed with B10.A mice. The heterozygous mice from these crosses were backcrossed to B10.A or B10 mice respectively. In total, 4069 backcross progeny were typed for their *H-2K* and *Aβ* genes by the microcytotoxicity test with peripheral lymphocytes and monoclonal antibodies against the respective antigens. The results are summarized in Table II. It appeared that three recombinants that carried the *wm7*-derived segment in the region proximal to the hotspot displayed high frequencies of recombination similar to those observed in the crosses between the *wm7* haplotype and laboratory haplotypes. Moreover, this high rate of recombination was observed not only in female but also in male meiosis. By contrast, no recombinants were identified in either female or male meiosis in the backcross progeny generated from B10A(R201) and B10(R231).

Pattern of distribution of recombinational breakpoints in the secondary recombinants

In order to map the recombinational breakpoints in the secondary recombinants, a total of 34 homozygous lines for these recombinant haplotypes were established by inter-crosses of the heterozygous mice. The DNAs containing the $A\beta_3/A\beta_2$ hotspot were amplified by PCR from the genomic DNAs of these mouse lines. We also prepared seven pairs of synthetic oligonucleotides that hybridize at various sites where there are polymorphic nucleotide substitutions between the wm7-derived recombinants and B10 strain. Either of the oligonucleotides from each pair had full-match sequence of one of the parental strains and had few mismatch sequences when compared with another parental strain. Using these pairs of oligonucleotides, dot hybridization was carried out for the amplified DNAs from the secondary recombinants. Washing hybridization filters at an appropriate dissociation temperature allowed us to distinguish the two parental sequences at the corresponding sites and to define the origin of the sites in the recombinant chromosomes. As summarized in Figure 2(C), it was clear that all breakpoints of the secondary recombinants were located in the amplified fragments, with the exception of SR8-1. Furthermore, most of them were located within a fragment between positions 837 and 1649, in which 11 out of 12 original recombinants were confined.

Table I. Origin of the MHC alleles in inbred and recombinant mice used.

Strain	Genetic locus				
	K	*Aβ*	*Eβ*	*S*	*D*
C57BL/10J	b	b	b	b	b
B10.A	k	k	k	d	d
B10.BR	k	k	k	k	k
B10.A(R201)	k |	wm7	wm7	wm7	wm7
B10.A(R206)	wm7 | k	k	d	d	
B10.A(R207)	wm7 | k	k	d	d	
B10.A(R209)	wm7 | k	k	d	d	
B10.A(R214)	wm7 | k	k	d	d	
B10.BR(R221)	wm7 |	k	k	k	k
B10.BR(R223)	wm7 |	k	k	k	k
B10.BR(R228)	wm7 |	k	k	k	k
B10(R225)	wm7 |	b	b	b	b
B10(R231)	b |	wm7	wm7	wm7	wm7
B10(R233)	b |	wm7	wm7	wm7	wm7
B10(R236)	b |	wm7	wm7	wm7	wm7

Vertical bars indicate the position of recombination.

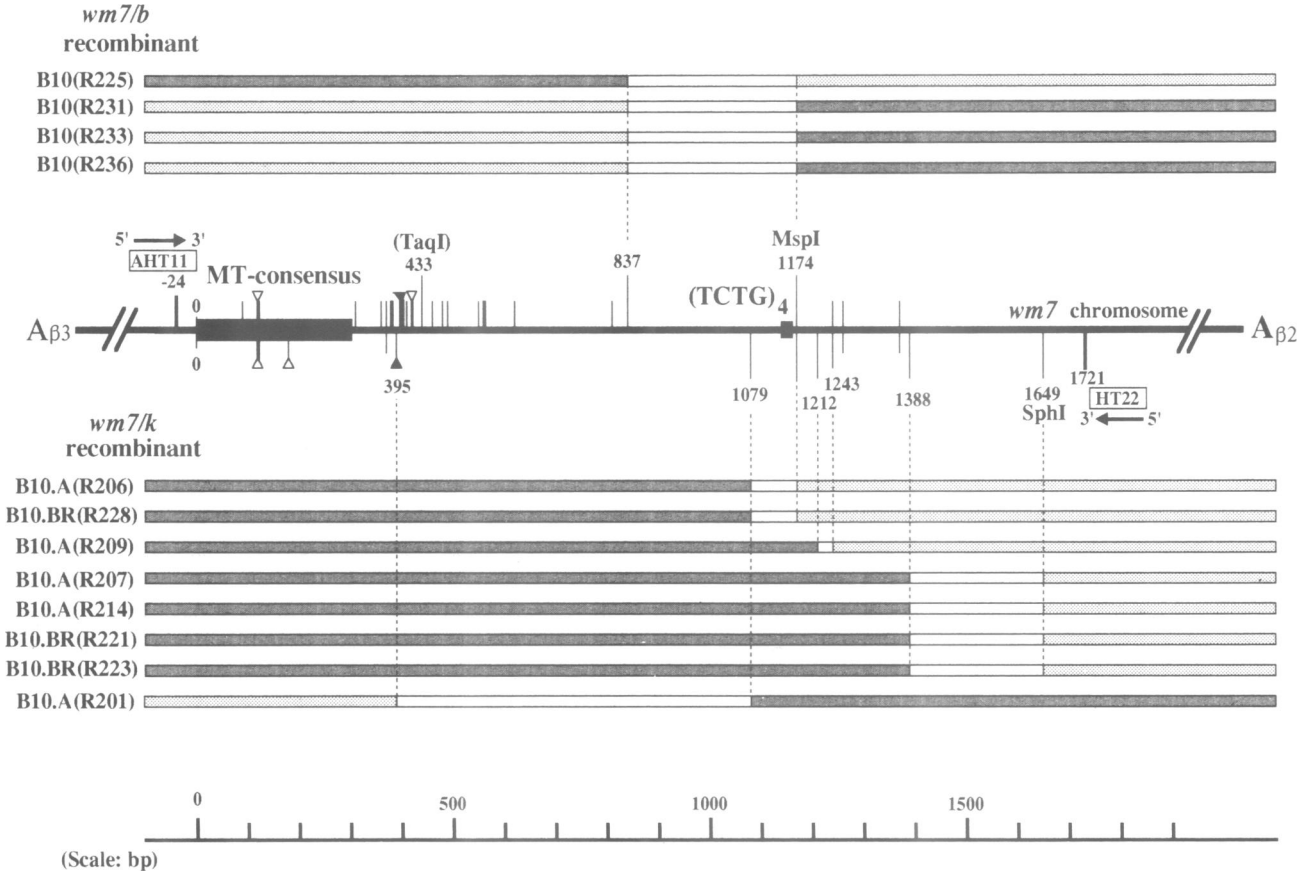

Fig. 1. Recombinational breakpoints in the cross between the *wm7* and laboratory haplotypes. DNA fragments from 12 independent recombinants from the crosses between the *wm7* haplotype and laboratory *b*, *k* and *a* haplotypes were amplified by PCR. A comparison of the sequences between the recombinant and the two parents allowed us to define the origin of sites with polymorphic nucleotide substitutions. The thick horizontal line indicates the *wm7* chromosome, on which the location of the MT-consensus sequence, the TCTG repeats and representative restriction sites are shown. The recombinants between the *wm7* and *b* haplotype are on the upper side from the wm7 chromosome, and recombinants between the wm7 and either the *k* or the *a* haplotype are on the lower side. Chromosomal segments derived from the wm7 and laboratory haplotypes in recombinants are indicated by hatched box and dotted box. Polymorphic nucleotide substitutions between the *wm7* and *b* haplotypes are indicated by bars that extend upwards from the wm7 chromosome, and those between the *wm7* and *k* or *a* haplotypes are indicated by bars that extend downwards from the wm7 chromosome. White and black triangles show deletions and insertions of nucleotides in the laboratory haplotype as compared with the *wm7* sequence. Numbers on the *wm7* chromosome represent polymorphic nucleotide positions, from the proximal end of the MT-consensus sequence, which mark the proximal and distal limits of the recombinational breakpoints in each recombinant. The breakpoints of the recombination are located somewhere within the empty boxes. Because there are no polymorphic nucleotide substitutions within an empty box, each box indicates the maximum length within which the breakpoints are located.

Table II. $K-A\beta$ recombination in the genetic crosses between the *wm7*-derived recombinants and laboratory strains.

Heterozygous parent	No. of secondary recombinants/No. of mice screened (% frequency ± SE)	
	Female	Male
B10.MOL-SGR[a] × B10, B10.A	15/743 (2.0 ± 0.5)	1/650 (0.2 ± 0.1)
B10.A(R206) × B10	6/362 (1.6 ± 0.6)	9/620 (1.4 ± 0.4)
B10.BR(R228) × B10	n.t.[b]	9/865 (1.0 ± 0.4)
B10.A(R209) × B10	9/319 (2.8 ± 0.9)	7/307 (2.2 ± 0.8)
B10.A(R201) × B10	0/394	0/482
B10(R231) × B10.A	0/373	0/347

[a]B10.H-2 congenic strain carrying the *wm7* haplotype.
[b]not tested.

Discussion

Direct sequencing of the 12 independent recombinants in the present study showed no evidence of either insertion or deletion of nucleotides in the fragments around the recombinational breakpoints. It has been reported that two recombinations that involved the *CAS3* haplotype similarly displayed neither insertion nor deletion of nucleotides (Uematsu *et al.*, 1986). Therefore, strand exchanges must have occurred at exactly homologous sites at the nucleotide level in the recombination at the $A\beta_3/A\beta_2$ hotspot. Until now, there have been no systematic studies designed to analyze the sites of recombination at the molecular level in mammalian meiosis. Thus, it appeared that recombination

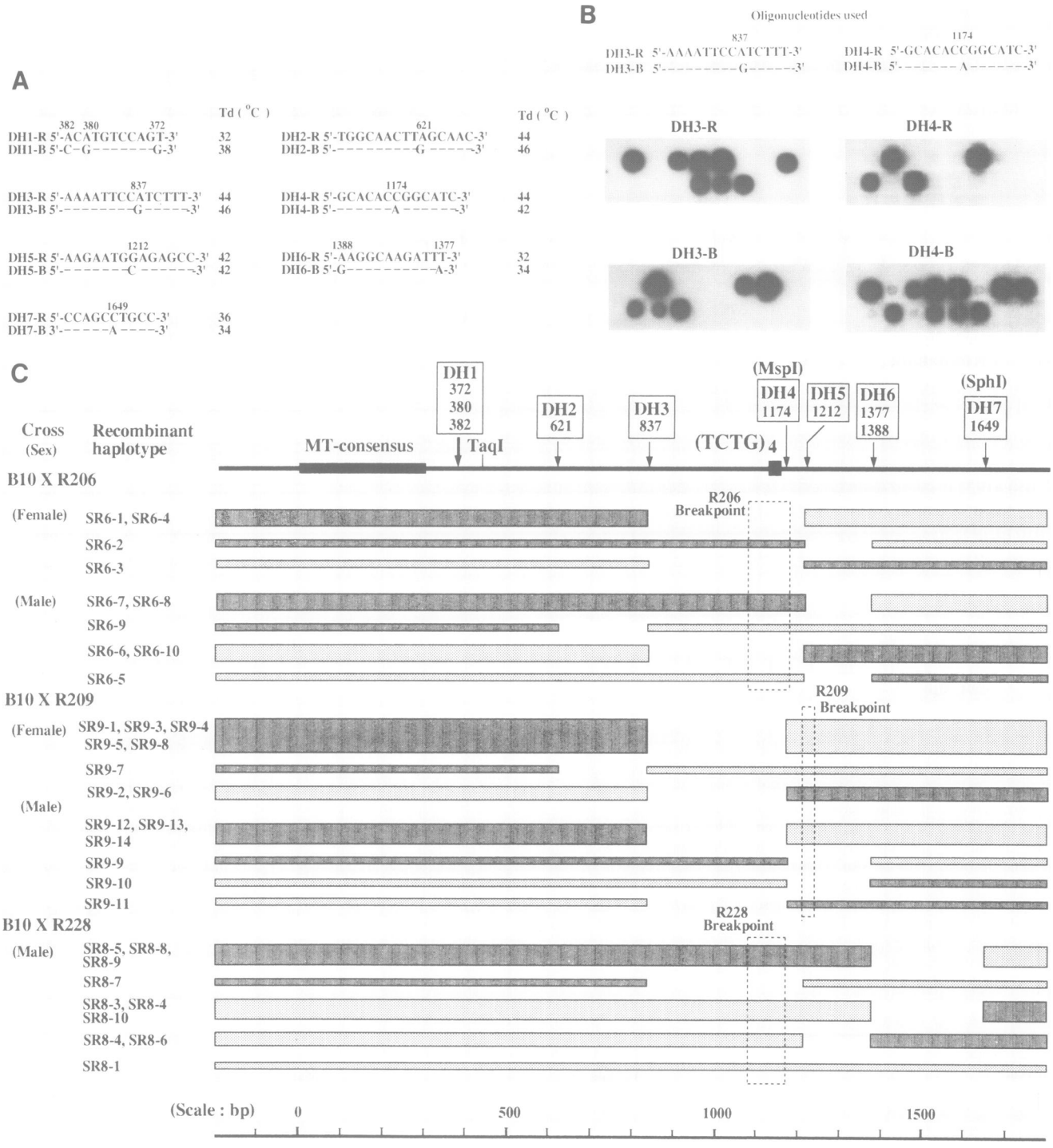

Fig. 2. Distribution of recombinational breakpoints in the secondary recombination. **(A)** Synthetic oligonucleotides used in the dot hybridizations. Either one of each pair of oligonucleotides, DH-R or DH-B, has a fully matched sequence to the recombinant or B10 strain, respectively. The polymorphic nucleotide positions are indicated by numbers above the oligonucleotides. **(B)** 100 ng of PCR-amplified DNAs from 14 secondary recombinants from the crosses between B10.A(R209) and C57BL/10J strains were hybridized with two pairs of ^{32}P-labeled synthetic oligonucleotides, DH3-R/DH3-B and DH4-R/DH4-B. **(C)** Distribution of the breakpoints in the 34 independent secondary recombinants. The breakpoints were defined based on the parental origin at marker sites hybridized by the seven pairs of synthetic oligonucleotides. Designations of each pair of oligonucleotides and the polymorphic sites are indicated in the boxes on top of the genomic structure of the hotspot. Chromosomal segments derived from the recombinants and the laboratory haplotypes are indicated by hatched boxes and dotted boxes. The thickness of the chromosomal segment represents the number of recombinants that have the same genotype and the same location of the breakpoints. The breakpoints in the original recombinants are surrounded by dotted lines.

between the two homologous chromosomes in mammalian meiosis has high fidelity.

The distribution of the breakpoints in the secondary recombination was very similar to that in the original recombination, and most breakpoints were confined within the segment from positions 837−1649. The breakpoints seemed to be clustered around the TCTG repeated sequence. The overall frequency of secondary recombination between

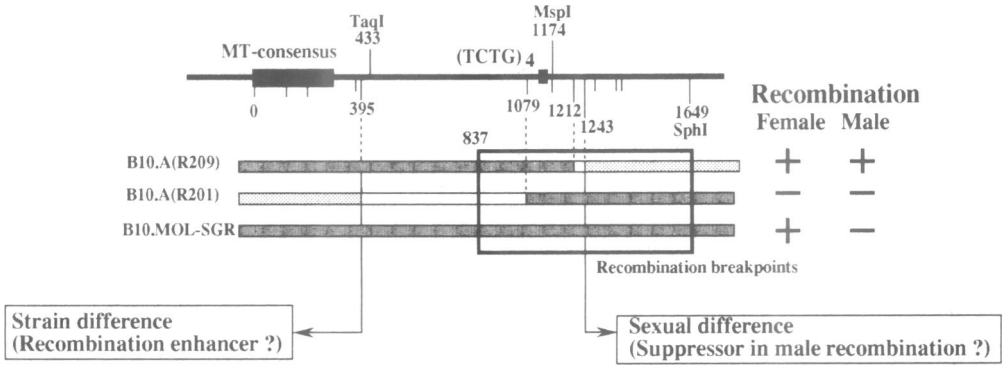

Fig. 3. Genetic control of sex-dependent meiotic recombination at the $A\beta_3/A\beta_2$ hotspot. Chromosomal organization of the two most informative recombinants and the parental B10.MOL-SGR ($H\text{-}2^{wm7}$) are indicated together with their capacities for enhancement of recombination in female and male meiosis. Distribution of the recombination breakpoints is indicated by the solid box. The genetic elements that control the strain-specific difference and sex-dependence are located in segment proximal to position 395 and in the segment distal to position 1243, respectively (see text).

positions 837 and 1649 is estimated to be >1.3% (31 recombinants out of 2473 backcross progeny screened). On the assumption that the total number of recombination units in the mouse chromosomes is 1600 cM and the size of the haploid genome is 3×10^9 bp, the rate of recombination appeared to be 3×10^3-fold higher than expected. The sharply defined distribution of the breakpoints suggests a presence of a target sequence that determines the site-specificity of the recombination. The two different hotspots characterized so far, that are located within the *Eβ* gene and in the $A\beta_3/A\beta_2$ region, have two common molecular elements: a 300 bp consensus sequence of the mouse middle repetitive MT-family, and repeated CCTG or its related TCTG tetramers, separated by a 1 kb of non-homologous DNA. In both hotspots, length polymorphism in the tetrameric repeated sequences, which may possibly be caused by unequal recombination, was observed (Kobori *et al.*, 1986; Uematsu *et al.*, 1986). Although there is no direct evidence in support of the crucial role, if any, of these elements in recombination, these two elements, and in particular the repeated TCTG sequence might serve as a signal for determining the site-specificity of the recombination at the $A\beta_3/A\beta_2$ hotspot.

From the present study it became clear that enhancement of recombination does not require the entire length of the hotspot from the *Taq*I site (position 433) to the *Sph*I site (position 1649). Two recombinants B10.A(R206) and B10.A(R209), in which the region distal to position 1174 or to position 1243 is replaced by the laboratory *a* haplotype, still showed the same rate of recombination as the rate observed in the cross between the *wm7* and laboratory haplotypes. In contrast to the above two recombinants, recombinant B10.A(R201) in which the region proximal to position 395 is replaced by the laboratory *a* haplotype showed no recombination in either female or male meiosis. The same result was obtained in the recombination between strains B10(R231) and B10.A. These data indicate that the *wm7* chromosome proximal to position 1174 is sufficient to stimulate the recombination. When the sequences are compared between the *wm7* and *k* haplotypes, few nucleotide substitutions are apparent especially in the region from position 395 to position 1174. Only a single nucleotide substitution, at position 1079, is observed. Since B10.A(R201), whose nucleotide at position 1079 originated from *wm7*, displayed no recombination, the genetic element that controls the level of recombination must reside in the region proximal to position 395. 42 out of 46 independent recombinants examined in the present study had their breakpoints within a segment of DNA from positions 837–1649. Hence, the present mating experiment demonstrated that strain-specific differences in the rate of recombination are controlled by an element physically separated from the recombinational breakpoints (see Figure 3). Because all the mice that we used were MHC congenic strains whose genetic backgrounds were replaced by the C57BL/10J background, the element under discussion should be linked to the MHC on chromosome 17. Further mating studies using recombinants at various sites in the region proximal to the hotspot may provide a more precise assignment.

At present, it is open to question whether the element that controls the level of recombination is a *cis*-acting factor or a *trans*-acting factor. In prokaryotes, *cis*-acting elements that enhance site-specific recombination have been well characterized. The *Chi* hotspot in λ phage is active only in the presence of distant cos sites located in *cis* and in proper orientation to the *Chi* sequence (Smith, 1983). Another example is known in the gene for flagellin in *Salmonella*. An enhancer element in the *hin* gene (recombinase gene) is required for a high level of site-specific recombination in the gene for flagellin. The enhancer must be present on the same DNA as the recombination sites but located some distance away from the sites (Johnson and Simon, 1987). Such a *cis*-acting enhancer of recombination has not been reported in any eukaryote genome. If the distant element at the $A\beta_3/A\beta_2$ hotspot works as a *cis*-acting factor, it would be the first example of a mammalian enhancer of recombination. Alternatively, if the element acts as a *trans*-acting factor, it may possibly encode some of the components involved in the recombination machinery, for example, a recombinase or its acccessary proteins.

It should be noted that, in the present mating experiments, the high rate of recombination was observed even in male meiosis when the same genetic cross showed a high frequency of recombination in female meiosis. As shown in Table II, the levels of male recombination were similar to those observed in female recombination. Thus, absence of recombination in male meiosis cannot be a characteristic directly associated with the hotspot. The factor that controls male recombination could be genetically separated from the target signal that determines the site-specificity of the recombination. This factor seems to reside in the chromosomal segment distal to position 1243, which marks

the distal limit of the breakpoint in recombinant B10.A(R209) (see Figure 3). In the *wm7* haplotype, this factor is thought to function as a suppressor of recombination in male meiosis. Further analysis of this factor may provide some clue towards an understanding of the general mechanism of the sex-related differences in recombination in mammalian chromosomes.

Materials and methods

Mouse strains

C57BL/10J (abbreviated as B10) and B10.A/J were purchased from the Jackson Laboratory, Bar Harbor, Maine and bred at the National Institute of Genetics (NIG), Mishima, Japan. All $A\beta_3/A\beta_2$ recombinants from the crosses between the *wm7* and either the *a*, *k* or *b* haplotypes were established and maintained at NIG.

Screening of recombinants between the K and Aβ genes

Heterozygous mice of the *wm7*-derived recombinants and laboratory strains were backcrossed to the B10 or B10.A strain. The backcross progeny were typed by the microcytotoxicity test with appropriate monoclonal antibodies which reacted with determinants specific for the K and Aβ antigens (Shiroishi *et al.*, 1987, 1990). Recombinants identified were confirmed by progeny test, and homozygous lines were established by inter-crossing of the offspring that were heterozygous for the recombinant haplotypes. In all, 34 lines were finally established. Several lines were extinguished before they were fully established.

PCR-amplification of genomic DNAs and direct sequencing

Genomic DNA (0.5 μg) was amplified by the standard polymerase chain reaction (Saiki, 1989) for 30 cycles using *Taq* DNA polymerase (Cetus Corp., CA, USA) and a pair of primers: AHT11,5′-ATCTTGAGAGGATGGCTTA-3′; and HT22,5′-TGAGAACTCTGGATAGGAT-3′ (see Figure 1), with 2 min extensions at 71°C, 1.0 min denaturations at 94°C, and 1.0 min primer annealing at 55°C. The amplified DNAs which were ~1.7 kb in size were subjected to electrophoresis on 0.7% agarose gel. The predominant DNA bands were cut out of the gel and the DNAs were purified by glass bead absorption and dissolved in TE (10 mM Tris−HCl, pH 7.5, 0.1 mM EDTA). Each reaction yielded ~100−200 ng of DNA, 20 ng of which were used in the secondary PCR reaction, with either the AHT11 or the HT22 primer, to amplify single-stranded DNA of the fragment. The DNA was purified by the same procedure as used in the primary amplification. About half of the purified product was used for each sequencing reaction. Sequencing was carried out by the standard method using the commercial kit, Sequenase (US Biochemicals, Cleveland, OH) and 12 synthesized oligonucleotides as primers, as described previously (Shiroishi *et al.*, 1990).

Dot hybridization

An aliquot of 100 ng of the PCR amplified DNA from each recombinant genome, with AHT11 and HT22 oligonucleotides used as primers, was blotted onto nylon membrane filter (Hybond-N, Amersham). After denaturation with a solution of 1.5 M NaCl and 0.5 M NaOH for 1 min and neutralization with 1 M Tris−HCl (pH 7.5) and 1.5 M NaCl for 1 min, the filter was hybridized with a battery of ^{32}P-labeled synthesized oligonucleotides in hybridization solution (6 × SSC, 5 × Denhardt's solution and 0.5% SDS) at room temperature for 1 h. The filter was then washed at the dissociation temperature appropriate for each oligonucleotide. The dissociation temperature, Td, was calculated as: Td = 4 × (number of G/C pairs) + 2 × (number of A/T pairs) (Suggs *et al.*, 1981). The dried filters were exposed to X-ray film (Kodak X-AR5).

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References

Begovich,A.B. and Jones,P. (1985) *Immunogenetics*, **22**, 523−532.
Davisson,M.T. and Roderick,T.H. (1981) In Green,M.C. (ed.), *Genetic Variants and Strains of the Laboratory Mouse*. Fisher, Stuttgart, pp. 283−313.
Donis-Keller,H. *et al.* (1987) *Cell*, **51**, 319−337.
Fischer Lindahl,K., Hausmann,B. and Chapman,V.M. (1983) *Nature*, **306**, 383−385.
Fischer Lindahl,K. (1986) *Curr. Top. Microbiol. Immunol.*, **127**, 272−278.
Heinlein,U.A.O., Lange-Sablitzy,R., Schaal,H. and Wille,W. (1986) *Nucleic Acids Res.*, **14**, 6403−6416.
Johnson,R.C. and Simon,M.I. (1987) *Trends Genet.*, **3**, 262−267.
Kobori,J.A., Strauss,E., Minard,K. and Hood,L. (1986) *Science*, **234**, 173−179.
Lafuse,W.P. and David,C.S. (1986) *Immunogenetics*, **24**, 352−360.
Lafuse,W.P., Savariarayan,S. and David,C.S. (1986) *J. Exp. Med.*, **163**, 1518−1528.
Reeves,R.H., Crowley,M.R., Ohara,B.F. and Gearhart,J.D. (1990) *Genomics*, **8**, 141−148.
Saha,B.K., Raziuddin and Cullen,S.E. (1986) *J. Immunol.*, **137**, 4004−4009.
Saiki,R.A. (1989) In Erlich,H.A. (ed.), *PCR Technology*, Stockton Press, pp. 7−22.
Shiroishi,T., Sagai,T. and Moriwaki,K. (1982) *Nature*, **300**, 370−372.
Shiroishi,T., Sagai,T. and Moriwaki,K. (1987) *Immunogenetics*, **25**, 258−262.
Shiroishi,T., Hanzawa,N., Sagai,T., Ishiura,M., Gojobori,T., Steinmetz,M. and Moriwaki,K. (1990) *Immunogenetics*, **31**, 79−88.
Smith,G.R. (1983) In Hendrix,R.W., Roberts,J.W., Stahl,F.W. and Weisberg,R.A. (eds), *Lambda II*. Cold Spring Harbor Laboratory Press, New York. pp. 175−209.
Steinmetz,M., Minard,K., Horvath,S. and Hood,L. (1982) *Nature*, **300**, 35−42.
Steinmetz,M., Stephan,D. and Fischer Lindahl,K. (1986) *Cell*, **64**, 895−904.
Steinmetz,M., Uematsu,Y. and Fischer Lindahl,K. (1987) *Trends Genet.*, **3**, 7−10.
Suggs,S.V., Hirose,T., Miyake,T., Kawashima,E.H., Johnson,M.J., Itakura,K. and Wallace,R.B. (1981) In Brown,D. and Fox,C.F. (eds), *Developmental Biology Using Purified Genes*. Academic Press, New York, pp. 683−693.
Uematsu,Y., Kiefer,H., Schulze,R., Fischer Lindahl,K. and Steinmetz,M. (1986) *EMBO J.*, **5**, 2123−2129.
Zimmerer,E.J., Ogin,E., Schreffler,D.C. and Passmore,H.C. (1987) *Immunogenetics*, **25**, 274−276.

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