A Torrid Zone on Mouse Chromosome 1 Containing a Cluster of Recombinational Hotspots

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ABSTRACT

Within the 2.38-Mb *Ath1* region of mouse chromosome 1, 42 of 45 genetic crossovers from crosses between C57BL/6J (B6) and either C3H/HeJ (H) or *Mus spretus* (SPRET) occurred in four zones (A–D); zone A, 100 kb long, contained a cluster of at least four recombination hotspots. F₁ sperm assays indicate that within this “torrid zone” the most active hotspot (A3) can initiate recombination on H and SPRET but not B6 chromosomes. The A3 DNA sequence contains a (G/C)TTT repeat, long stretches of A or T, and a cyclic variation in AT content. Recombination was drastically reduced in a cross between B6 and a B6.SPRET *Ath1* congenic strain, but was unaffected in a B6 × B6.H *Ath1* congenic cross. Similar nonrandom clustering of hotspots has been observed in yeast and the major histocompatibility complexes of human and mouse. To the extent that torrid zones are a general feature of mammalian genomes, they have considerable implications for genetic mapping strategies in both human populations and mouse crosses.

IN mammals, higher plants, and yeast, meiotic crossovers tend to localize in chromosomal regions called hotspots (LICHTEN and GOLDMAN 1995; PETES 2001). This concentration of recombination in limited regions requires that there be corresponding “cold” regions of diminished or no recombination. The resulting “granularity” of genetic maps limits mapping resolution in experimental organisms. In humans, the existence of hotspots affects proposals to use linkage disequilibrium as a means of mapping genes important to human health and defines the boundaries of the disequilibrium segments (REICH *et al.* 2002; PHILLIPS *et al.* 2003).

Although more than a dozen individual mammalian hotspots have been identified, we know relatively little about their genome-wide organization. The only chromosome regions examined in detail have been the major histocompatibility complexes (MHCs) of mouse (SHIROISHI *et al.* 1995) and human (JEFFREYS *et al.* 2001) where hotspots account for the vast majority of recombination. In contrast, both regional (BAUDAT and NICOLAS 1997) and genome-wide (GERTON *et al.* 2000) maps of hotspots have been constructed for yeast.

Numerous studies have established several basic features of mammalian hotspots since they were first discovered in mouse (STEINMETZ *et al.* 1982) and human (CHAKRAVARTI *et al.* 1986). They are uniformly 1–2 kb in length (LICHTEN and GOLDMAN 1995; JEFFREYS *et al.* 2001; PETES 2001; BUCHNER *et al.* 2003), can be haplotype specific (LAFUSE and DAVID 1986; STEINMETZ *et al.*

1986), may be sex specific (SHIROISHI *et al.* 1990), and vary considerably in the intensity of crossing over. Hotspot recombination frequencies ranging from 0.01 to 2.0% in mice (BUCHNER *et al.* 2003) and as low as 0.0005% to as high as 1.7% in humans (DE MASSY 2003) have been reported. PITTMAN and SCHIMENTI (1998) have provided a detailed, useful summary of these issues.

The concentration of recombination within hotspots raises the question of whether any significant amount of truly random recombination exists; it may well be that apparently random recombination occurs at hotspots of such low intensity that they are difficult to detect in pedigree analyses.

In this report we define the recombination features of a non-MHC mammalian chromosome region by refining the locations of a set of 45 crossovers that arose in 6275 meioses in the 2.38-Mb region of mouse chromosome 1 surrounding the atherosclerosis susceptibility gene, *Ath1*. The *Ath1* gene lies between markers *D1Mit159* and *D1Mit398*, which are 2.38 Mb apart (PHELAN *et al.* 2002), and plays an important role in determining either resistance or susceptibility to atherosclerosis in mice (PAIGEN *et al.* 1987a,b,c; PHELAN *et al.* 2002).

The organization of recombination within the *Ath1* region strongly resembles that of the human and mouse MHC regions. Seven hotspots with varying recombination frequencies contain 42 of the 45 recombination sites. Four of these are concentrated in a “torrid zone” of 100 kb. The lack of useful markers made it impossible to tell whether one of the four clustered hotspots actually consists of two closely spaced hotspots; if so, that would bring the total to eight with five in the torrid

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TABLE 1
Mouse crosses

Cross	Parents	Type of cross	Recombining parent(s)	No. of animals	No. of tested chromosomes	No. of recombinants	cM
1	B6 × H F ₁	IC	Male + female	2139	4278	27	0.63
2	B6Smn.H- <i>Tnfsf6</i> ^{gld} F ₁	IC	Male + female	326	652	4	0.61
3	B6 × (B6 Smn. H Tn <i>tsf6</i> ^{gld}) F ₁	BC	Male only	653	653	4	0.61
4	(B6 × SPRET) F ₁ × B6	BC	Female only	692	692	10	1.45
5	B6 × (B6.SPRET × B6) F ₁	BC	Male only	1763	1763	4 ^a	0.23

IC, intercross; BC, backcross.

^a These four recombinants were not mapped to limiting resolution because the DNAs were no longer available.

zone. A similar clustering of hotspots has been reported for the MHC of humans (JEFFREYS *et al.* 2001; CULLEN *et al.* 2002) and mice (SHIROISHI *et al.* 1995), suggesting that torrid zones may be a common feature of mammalian genomes. Although the physical scale of recombination in yeast is vastly different from that in mammals, clustering of hotspots has been reported there as well (ZENVIRTH *et al.* 1992; BAUDAT and NICOLAS 1997; GER-TON *et al.* 2000).

MATERIALS AND METHODS

Mice: Mice from strains C57BL/6J (B6), C3H/HeJ(H), (B6 × C3H)F₁, SPRET/Eij (SPRET), CAST/Eij (CAST), and B6/Smn.C3H-*Tnfsf6*^{gld}, a B6 congenic strain containing a genomic segment derived from H surrounding the interval of interest, were obtained from The Jackson Laboratory (Bar Harbor, ME) and used to make five crosses (Table 1). These crosses were carried out to positionally clone *Ath1*, and the stored DNAs were used to define the recombinational hotspots. Cross 5 was made between B6 and an F₁ B6 × B6.SPRET-*Ath1*⁺ congenic strain heterozygous for the *Ath1* region from the SPRET strain. The construction of this congenic is described in PHELAN *et al.* (2002). Sperm assays were carried out on B6 × H and B6 × CAST F₁ males. All experiments were approved by the animal care and use committee of The Jackson Laboratory.

PCR and electrophoresis: Mouse DNA was extracted from tail tips. PCR was performed using standard conditions, with an annealing temperature of 55° for MIT microsatellite markers and an annealing temperature of 58° for custom-made oligonucleotides. Primers for MIT markers were purchased from Research Genetics (Huntsville, AL) and custom oligos were purchased from Ransom Hill Bioscience (Ramona, CA) and Integrated DNA Technologies (Coralville, IA). PCR products were resolved on 4% agarose gels.

SNPs and DNA sequence analysis: Single nucleotide polymorphisms (SNPs) among the three strains were identified by sequencing genomic DNA. We determined 10.9 kb of sequence for B6 and H and 6.3 kb for SPRET. Primers flanking individual SNPs were designed and used to amplify genomic segments for sequence analysis to determine the genotypes of recombinant animals. These new markers, their locations, and the primer pairs used to detect them are listed in Table 2. The Microchemistry Service of The Jackson Laboratory sequenced PCR products using Big Dye Terminator cycle sequencing chemistry on an Applied Biosystems 3700 (Foster

City, CA). Sequences were analyzed with Sequencher v.4.0.5 (Gene Codes, Ann Arbor, MI).

Sperm recombination assay: The recombination assay was carried out in two rounds of allele-specific PCR. Four SNPs distinguishing each strain combination were selected, and allele-specific primers having the polymorphic nucleotide at their 3'-end were designed for each SNP. The locations of the two forward and two reverse primers are indicated in Figure 2. In the first round, a 937-bp fragment was amplified for 30 cycles using pairs of strain-specific primers for B6 *vs.* H, and a 900-bp fragment was amplified with pairs of strain-specific primers for B6 *vs.* CAST. For some reagents concentrations are expressed relative to the proprietary kits provided by the companies. The reaction mixtures contained 1× titanium buffer, 0.2 mM dNTPs, 0.2 μM each primer and 0.2× titanium Taq (BD Clontech, Palo Alto, CA), and 20 ng genomic DNA. The product was diluted 500 times and used as a template for the second round of PCR, which was carried out in a real-time format with allele-specific primers nested to those used in the first PCR. The reaction mixtures contained 1× titanium buffer, 1× reference dye (Sigma, St. Louis; R4526), 0.1 mM dNTPs, 0.2× SYBR green (Molecular Probes, Eugene, OR), 0.25 mM betaine (Sigma), 0.1 μM each primer, and 0.2× titanium Taq. The expected length of the product for B6-H analysis was 748 bp and for B6-CAST analysis was 673 bp. The product specificity in the second round was confirmed by melting curve analysis. All possible allele-specific combinations were tested.

In each case, six F₁ sperm DNA samples were tested, as well as DNA samples of the original strains and 1:1 mixture of both as controls.

The following allele-specific primers were used for distinguishing B6 and H sequences:

B6-forward 1—CAAGAACACAGTTTCACTGAACCA
 B6-reverse 1—TGGGTGTGGCGCATGG
 H-forward 1—CAAGAACACAGTTTCACTGAACCG
 H-reverse 1—TGGGTGTGGCGAATATGA
 B6-forward 2—GCCCCTCTCTTGACTTGAGG
 B6-reverse 2—TAGCAGCTTCTGGACATGGTC
 H-forward 2—GCCCCTCTCTTGACTTGAGC
 H-reverse 2—GGCAGCTTCTGGACATGGTA

For distinguishing B6 *vs.* CAST, the same forward primers were used because CAST has the same alleles as H. The reverse primers were:

B6-reverse 3—GGTCAGGGCACGTCTGTGAC
 B6-reverse 4—GCTCAAGGTCAGTGTGGATTGT
 CAST-reverse 3—GGTCAGGGCACGTCTGTGAT
 CAST-reverse 4—GCTCAAGGTCAGTGTGGATTGC

TABLE 2
Markers used in this study

Official name	Position (bp)	Position (bp)	Sequence	Origin of sequence	Amplicon	Polymorphic B6 × C3H	Polymorphic B6 × Spret
D1 Pgn 1	160747597	163636502	F-CTGCTTGACTTGGTGCTTTC R-TATTCTCCCCTGGTTTAGGC	Celera	GA repeat	Yes	Yes
D1 Pgn 2	162640072	165551352	F-GGCAGGTTGAAATGATTCAG R-CAGATAGCTTTTGACCGAGAATG	Celera/Ensembl	CA repeat	Yes	No
D1 Pgn 3	160675317	163564221	F-GTACAGTTTCTTTTGTGGG R-TTTTGGCTGATGACACACAC	Celera/Ensembl	GCTTT repeat	Yes	?
D1 Pgn 4	160685903	163574808	F-AATAGGACATTAGGTGGGG R-TCTATGAATTTGAGGCCAGC	Celera	AT and CA repeat	Yes	?
D1 Pgn 5	162692173	165594644	F-GATTGCATAGTGGGACGAG R-TGAACCTTGCTTCAATTATCTGTG	Celera/Ensembl	CT and CA repeat	Yes	?
D1 Pgn 6	160651618	163539842	F-AAAGCACCTACAGGCATCTG R-TTGATAAGTCTAGCCTTCCTTC	Celera/Ensembl	CA repeat	No	Yes
D1 Pgn 7	160672675	163561580	F-CAGTGTCCAAAGTGCTGATG R-TCTCTATGCCATTGGAAAG	Celera/Ensembl	GTTT repeat	No	Yes
D1 Pgn 8	160687077	163575982	F-TCCCTCAGTGAATGTGATTTC R-TGCTTCAAAATGTGCAAAAC	Celera/Ensembl	CA repeat	Yes	No
D1 Pgn 9	160631567	163520073	F-GGTGTCCACTGCTCAATAC R-ACCTTCTGGGCTAACATTCC	Celera/Ensembl	CA and GA repeat	Yes	Yes
D1 Pgn 10	160635799	163524305	F-AAGTGAGCCCTGTGAAAATG R-CCCAAGGACACTTCATATCG	Celera/Ensembl	GTT Repeat	Yes	?
D1 Pgn 11	160708827	163597732	F-TACCCCTCGCATCTCTGAAAAG R-CGGCACATGACAAACTACAG	Celera/Ensembl	CA and GA repeat	Yes	Yes
D1 Pgn 12	160732227	163621132	F-CAGCTTTTTCACCACGTTTTTC R-TTCAAGGGCTACTGAAAGCTG	Celera/Ensembl	CA and GA repeat	Yes	Yes
Official name	Position (bp)	Position (bp)	Sequence (20 bp on either side of SNP)	B6	C3H	Spretus	
D1 Pgn 13	160670965	163559870	CCATCTATTGTTCCTTTGT(Y)CCTT TTCTTCTCACCATTTC	C	T		
D1 Pgn 14	160675800	163564705	GCCCACTCTCTGACTTGAG(S)TCTC CAGGCTCAGCATCTT	G	C	C	
D1 Pgn 15	160675807	163564712	CTCTTGACTTGAGGTCTCCA(M)GCC TCAGCATCTTCATCCAT	C	C	A	
D1 Pgn 16	160676252	163565157	ACCATCTCTCCAGCCCCACT(R)ATTG TGATTTTAAATATAA	A	A	G	
D1 Pgn 17	160676343	163565248	GTGTGAAAGAAAACAAAAC(R)ACAA CAAAACAGCAAGTTA	A	A	G	
D1 Pgn 18	160676527	163565432	TGGAGTCACAGACGTGCCCT(K)AC CATGTCCAGAAAGCTGCTA	G	T	T	

(continued)

TABLE 2
(Continued)

Official name	Position (bp)	Position (bp)	Sequence (20 bp on either side of SNP)	B6	C3H	Spretus
D1 Pgn 19	160676585	163565490	AGCCACACCCACAGGAAGCC(K) ATCACACAGTCTGTCTCTTG	G	G	T
D1 Pgn 20	160676633	163565538	CATGCCCTTGCTCTAGACATC(R)TT CCAATCGCCTATGTCTCTG	G	G	A
D1 Pgn 21	160676883	163565788	TGGCAATTTGTTCTGTGCCAT(N)GCT GACACATAGCTTTGGGC	C	C	T
D1 Pgn 22	160676346-8	163565251-3	TGAAAGAAAAACAACAAAC(AAC)A AACCAGCAAAAGTTAAATTGT	In	In	Del
D1 Pgn 23	160676419-22	163565324-7	TCTTTGTTGTTTGTGTTT(GTTT) GTTTTCATTTGTTTGAAA	In	In	Del
D1 Pgn 24	160676566-77	163565473-84	CCATGTTGCGCCACACCCACA(GCCA CACCCACA)GGAAGCCGATCACACA GTCT	In	Del	Del

Positions are given for Ensembl release 32.

RESULTS AND DISCUSSION

Recombination frequencies: *Ath1* resides in a 2.38-Mb region (Ensembl Mouse Release 32) between proximal marker *D1Mit159* and distal marker *D1Mit398* (Phelan *et al.* 2002). Marker *D1Pgn5*, which is <2 kb away, was used instead of *D1Mit398* for crosses involving H because *D1Mit398* could not be amplified from H DNA. The recombination frequencies observed across this region in various crosses are presented in Table 1.

To compare recombination frequencies in the various crosses we used Fisher's exact *t*-test as well as a two-tailed *Z*-test to estimate the probability that two ratios are or are not drawn from the same population. There is <5% probability that any of the first three crosses, involving B6 and H, differ one from another. From this, it appears that making the remainder of chromosome 1 homozygous B6, as in cross 2, had little effect. Similarly, it is unlikely that sex had any influence on recombination (cross 3 compared to crosses 1 and/or 2).

Combining the data from all three B6 × H crosses gives 35 recombinants among 5583 tested chromosomes for a genetic distance of 0.63 cM. In the cross between B6 and SPRET (cross 4), the frequency of recombination across the *Ath1* region was greater, 1.45 cM (*P* = <0.02). These overall frequencies correspond to 0.26 and 0.61 cM/Mb and are comparable to the mouse genome-wide average of 0.55 cM/Mb (1485 cM/2700 Mb).

Loss of recombination in SPRET congenic strain: When compared with the B6 × SPRET cross 4, recombination across the *Ath1* region was greatly reduced in the B6 × B6.SPRET cross 5 in which the rest of the genome of the recombining parent was homozygous B6 (*P* < 0.0005). Several explanations for this observation are possible: (1) recombination occurs preferentially in regions of exact pairing, which reduces recombination across the *Ath1* region in the B6 × B6.SPRET cross because the remainder of the chromosome is now homozygous for B6 sequences (this effect would be expected to be much greater with SPRET than with C3H because of the much greater evolutionary divergence of SPRET); (2) sex strongly influences recombination across this region in B6 × SPRET crosses but not in B6 × H crosses; or (3) the existence of a hotspot ultimately depends on recognition by the product of an unlinked gene, the SPRET allele of which is absent from the congenic strain. Unfortunately, our data do not allow us to choose which of these possibilities is correct.

Fine mapping of crossover sites: The distribution of crossovers across the *Ath1* region in B6 × H crosses is distinctly nonrandom. To map their location, the Ensembl public mouse genome database (version 32) was used to design PCR primers flanking simple sequence length polymorphisms (SSLPs) and SNPs. The SSLPs were typed by electrophoresis mobility and the SNPs by sequencing across the polymorphic regions. Using these markers, 34 of the 35 B6 × H crossovers fell into four

FIGURE 1.—The location of the 45 crossovers mapped in the 2.38-Mb *Ath1* region. The first two rows present the location of all transcription units in the region with arrows describing the direction of transcription. In the third row the boxes A, B, C, and D are the regions containing multiple crossovers, with the numbers of crossovers indicated below each box; distances in kilobases are indicated above the line. The locations of the three crossovers mapping outside these regions, two from B6 $\times$ SPRET and one from B6 $\times$ H, are shown as dotted vertical lines. The fourth row is an expanded map of the A region showing the four subregions A1–A4. Again, distances are in kilobases and numbers of observed crossovers are below each box. The hatched area adjacent to A3 is the location of the additional possible hotspot subregion described in the text. The markers used to delineate the ends of the subregions are listed above the row. The fifth and sixth rows are expansions of the A3 hotspot, indicating numbers of crossovers occurring in each interval in B6 $\times$ H and B6 $\times$ SPRET crosses, along with the markers used to define the intervals. A base-pair scale is provided at the bottom.

zones, designated A–D (Figure 1), which includes only the markers delineating breakpoint locations). The A zone, 100.8 kb long, contained 20 crossovers, the 8.5-kb B zone contained 3 crossovers, the 35-kb C zone contained 9 crossovers, and the 14.9-kb D zone contained 2 crossovers. Efforts to locate the true boundaries of these zones were limited by the availability of markers, and their actual lengths could be significantly shorter.

The 29 crossovers in the A and C zones clearly result from nonrandom clustering of crossover sites. Do the B and D zones also represent sites of nonrandom clustering? Consider the B region first. Including a Bonferroni correction for multiple sites, the likelihood that 3 of the 6 remaining crossovers will fall within the same 8.5-kb interval is $\sim 20 \times (8.5/2380)^2$ or 0.00026, so the B zone is nonrandom. Similarly, the probability that 2 of the remaining 3 crossovers will fall into a 14.8-kb region is $\sim 3 \times 15/2380$ or 0.019, suggesting that the D zone is also the result of nonrandom clustering and likely to contain a hotspot.

In contrast to the recombinants from B6 $\times$ H crosses,

most (8 of 10) of the B6 $\times$ SPRET recombinants (cross 4) occurred in the A zone. The B, C, and D zones either were not active regions of recombination in crosses between B6 and SPRET or were not detected as such because of the smaller number of meioses tested. If the recombination frequencies for these regions in B6 $\times$ SPRET crosses were equivalent to those in B6 $\times$ H crosses, we would have expected only two crossovers distributed among three regions on the basis of the number of chromosomes examined, and their absence may simply have been due to chance.

The recombination zones were separated by long, entirely nonrecombinant stretches extending in length from >300 to nearly 1000 kb. The three singleton crossovers that did not map within the A–D zones, indicated by dotted vertical lines in the top row of Figure 1, were located close to zones A and B, suggesting that even their locations were not random.

We focused on the A region for further study. The B and D regions were the sites of few recombinations, and we were unable to find any sequence differences

TABLE 3
Results of sperm PCR assays

PCR-1 PCR-2	B—B B-B	H—H H-H	B—B H-H	H—H B-B	H—B B-H	H—B H-B
B6	21.8	None	None	None	None	None
C3H	None	20.6	None	None	None	None
B6 + C3H	22.4	26.9	None	None	None	None
Water	None	None	None	None	None	None
F1-1	23.1	26.3	None	None	None	28.0
F1-2	23.7	28.0	None	None	None	26.1
F1-3	23.3	22.2	None	None	None	23.6
F1-4	28.5	21.5	None	None	None	25.5
F1-5	23.3	24.2	None	None	None	25.4
F1-6	24.5	26.6	None	None	None	None

PCR-1 was run 30 cycles with the forward 1 and reverse 1 primers of Figure 2 using titanium Taq. The products were diluted 1:500 for PCR-2 with the forward 2 and reverse 2 primers of Figure 2, again with titanium Taq and betaine for 40 cycles in real-time format. The numbers in columns 2–7 are the cycle at which the signal exceeded the threshold value; these samples gave a T_m between 87.0° and 87.5°.

between B6 and H to fine map the C region. Random sequencing of 5 kb across the C region of both genomes failed to detect any sequence differences, suggesting that the B6 and H genomes share a common haplotype across this segment of chromosome 1.

Figure 1B describes the results of fine mapping the A zone. It contains at least four distinct hotspots (A1–A4) and possibly a fifth. Fine mapping of the A region recombinants arising in the B6 and H crosses was limited by the paucity of molecular markers in this region, and it was not possible to decide whether three of the B6 × H recombinants occurred within the A3 region or in an adjacent region that defined yet another hotspot.

The eight B6 × SPRET crossovers in the A zone describe a typical hotspot. All fell within a 1.1-kb interval, designated A3, between markers D1 Pgn 14 and D1 Pgn 21 (Figure 1B). Their locations within the A3 interval, mapped using all available markers, are shown in Figure 1C.

Six of the 20 A zone B6 × H crossovers fell within the 1.1-kb A3 interval, indicating that they result from activity of the same hotspot. Three additional B6 × H crossovers fell within an adjacent 9.2-kb interval (D1 Pgn 19–D1 Pgn 4) that could not be subdivided with available markers. Unable to resolve their locations further, we cannot say whether these recombinants belong to the A3 hotspot or another that is closely adjacent.

Recombination across the A3 hotspot was 7–11 times greater in B6 × SPRET than in B6 × H depending on whether the A3 hotspot contained six or nine crossovers. Thus, haplotype specificity appears to determine the activity as well as the location of individual hotspots.

Another six of the B6 × H crossovers fell in the 19.3-kb A2 region, apparently defining yet another hotspot as A2 and A3 are at least 4.7 kb apart.

Among the remaining crossovers, two fell in the A1 region and three in the A4 region, which are, respectively, 15.7 and 22.8 kb distant from A2 and A3. It is likely that A1 and A4 represent additional hotspots. Including Bonferroni corrections, the combined probability that two of the five remaining crossovers within the A zone fell by chance within one 4.4-kb interval and that the remaining three cluster within a 23.6-kb interval is only 0.024 [$10 \times (4.4/100.8) \times (23.6/100.8)^2$].

Strain specificity of hotspot origin: Nested PCR assays of DNA sequences in F₁ sperm from B6 × H and B6 × CAST crosses indicated that recombination at the A3 hotspot occurs in the first, but not the second cross, suggesting that the recombinants obtained in B6 × H and B6 × SPRET crosses initiated on H and SPRET chromosomes, but not on B6 chromosomes.

The validity of the assay was confirmed by comparing F₁ sperm DNA samples with sperm DNA samples of the original strains and 1:1 mixtures of the two parental sperm DNAs. When B6-specific primers were used in the first and second rounds of amplification, we detected specific amplification in the second round from B6 parental DNA, a mixture of B and H DNAs, and F₁ sperm DNA as expected, but not in the H sample (see Table 3). Equivalently specific results were obtained using H-specific primers in both rounds, detecting specific amplification in all samples but B6. Additionally, no specific amplification was detected from any sample when the first round used either B6- or H-specific primers and the second round used primers from the other strain or recombinant primers.

When recombinant primers were used in the first round (B6forward and Hreverse, or Hforward and B6reverse) and nested primers with the same strain combinations in the second round, specific signal was detected in five of six F₁ sperm samples, indicating recombination events in the region studied (Table 3). Using recombinant primers, no specific signal was detected in any of the control samples (B6, H, or B6 + H mixture).

The analysis of B6 × CAST F₁ hybrids produced different results. The appropriate amplicon-specific signal was detected using strain-specific primers with DNA from the same strain in both rounds, and the controls behaved as expected. However, there was no specific amplification using recombinant primers in two F₁ sperm samples tested six times each (data not shown), suggesting that recombination does not occur at this site between B6 and CAST chromosomes. We were unable to test B6 × SPRET F₁ males, as they are sterile, and were unable to test H × CAST F₁ males as the parents lack the sequence differences necessary for detecting recombination.

Given the recombinants obtained in the original crosses, we take the sperm data as evidence that the double-strand break initiating recombination at the A3 hotspot can occur on C3H and SPRET chromosomes, but not on the B6 chromosome. It seems unlikely that B6

DNA sequences and hotspot locations: There was no obvious correlation in the Ath1 region between the locations of hotspots and transcription units (indicated in Figure 1 in both directions). To date, no unique sequence identifiers for hotspots have been identified. Although tetra- or hexanucleotide repeats are sometimes found in hotspots, they are not universally present (BUCHNER *et al.* 2003). In yeast, PETES (2001) has sug-

gested that three classes of hotspots may exist. It is possible that the difficulty of finding a unique identifier in mammals reflects the existence of distinct families of hotspots, each with its own signature. Additionally, the elegant genetic experiments of SHIROISHI *et al.* (1991) demonstrated that recombination at the Ab3-Ab2 hotspot in the mouse *H2* complex depends on flanking DNA sequences; a region centromere proximal to the hotspot determines whether recombination will occur, and a region distal to the hotspot determines whether it can occur in male meioses.

Given the paucity of sequence information on mammalian hotspots and the possibility of multiple hotspot families, we have sequenced the A3 hotspot in B6, H, and SPRET (Figure 2) and find several notable features. Near the middle there is a 13-fold repeat in B6 and H and a 12-fold repeat in SPRET of the tetranucleotide TTT(G/C), with only slight sequence variation, thus adding to the number of hotspots containing short tandem repeats. Long stretches of A or T are present, contradicting the suggestion that hotspots represent regions of high GC content. And there is a marked cyclic variation in AT content (Figure 3), although we have not found this in other hotspots. If anything, these results support the notion that multiple families of hotspots may exist, each with their own identifier sequences. Additionally, the minimal sequence differences between all three haplotypes and especially between CAST and C3H, suggest that flanking sequences probably play a role in the activity of this hotspot.

Torrid zones: Regional clustering of hotspots has also been observed in the HLA region of human chromosome 6 (JEFFREYS *et al.* 2001; CULLEN *et al.* 2002) and the H2 region of mouse chromosome 17 (SHIROISHI *et al.* 1995). Because 40% of the genes in the major histocompatibility complex function in immune responses and recombination sites might well be under intense selection, finding torrid zones there might reflect the outcome of strong evolutionary forces. Our finding of hotspot clustering in the first other extended mammalian chromosome region to be examined, one not obviously subject to strong selective forces, suggests that torrid zones are a common feature of mammalian recombination. If so, their existence will further constrain the resolution of mammalian gene mapping, whether mouse or human, and it becomes important to understand their properties and distribution in the genome.

Whether or not there has been strong evolutionary selection for clustering of hotspots, the existence of a torrid zone suggests that some higher order feature of chromatin organization is involved. Although there is strong evidence that transcription factor binding, but not transcription itself, is related to recombination in yeast (PETES 2001), such a close association has not been observed in mammals. In this case the torrid zone is not gene rich and the most intense hotspot (A3) is 10 kb upstream of the nearest gene. Another possibility

FIGURE 3.—Percentage AT in 50-bp intervals of 36 DNA across the A3 hotspot. The curve is very little different for H or SPRET.

is that chromosome looping provides a framework for recombination, but there is presently no way to test for a relationship between looping and recombination. And finally, the explanation could reside in some presently unappreciated aspect of chromatin organization.

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