Trans-acting Factors Influence Several Mouse Meiotic Recombination Hotspots on Chromosome I

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Meiotic recombination is essential for maintenance of genome stability and genetic diversity in the offspring. Although the role of different proteins making up the recombination machinery is fairly well elucidated, much less is known about the distribution of recombination sites in mammals and the factors regulating it.

Trans-Acting Factors: To search for trans-acting factors controlling the positioning of recombination events, we compared high-resolution maps of telomere-proximal 25 Mb in an interstrain cross of C57BL/6JxCAST/EiJ and a congenic cross of B6xB6.CAST-1T, in which the distal half of Chr 1 from CAST/EiJ is transferred onto B6 background. In both crosses, F1 animals are heterozygous B6/CAST through the distal half of Chr.1, however, only the F1 from the interstrain cross possess CAST alleles in the rest of the genome. The lack of CAST alleles in the congenic cross resulted in loss of crossing over at specific hotspots as well as activation of previously undetected hotspots, suggesting the presence of distant trans-acting genes whose CAST allele activates or suppresses the activity of specific hotspots.

Site of Action: The trans-acting genes may control different steps in the recombination pathway, at the initiation or at later checkpoints, leading to crossovers or noncrossovers. To address this question, we developed a novel sperm assay, which allows detection and discrimination between crossover and noncrossover events and the localization along the hotspot region. Both crossover and conversion events were detected at two hotspots in the sperm of the interstrain F1 males but none in the congenic F1, whose progeny also lacks recombination at these sites. This result indicates that the trans-acting genes control the initiation steps of recombination.

Mapping Trans-Acting Genes: For the purpose of mapping trans-acting genes affecting recombination, we set an F2 cross of C57BL/6J x CAST/EiJ. Sperm DNA from all F2 males heterozygous at the 25 Mb telomere-proximal region of Chr.1 was tested using a two-round PCR assay to detect crossover events at specific hotspots. Our first results show involvement of more than one gene in the hotspot regulation, and are most consistent with the involvement of one gene common for all three tested hotspots, and additional genes specific for each hotspot.

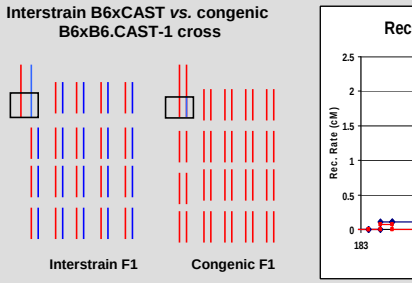

Fig.1. In F1 of the interstrain cross CAST alleles are present on all chromosomes. In congenic F1 CAST alleles are present only in the congenic region, the rest of the genome is homozygous C57BL/6J. Boxes indicate the region of recombination mapping.

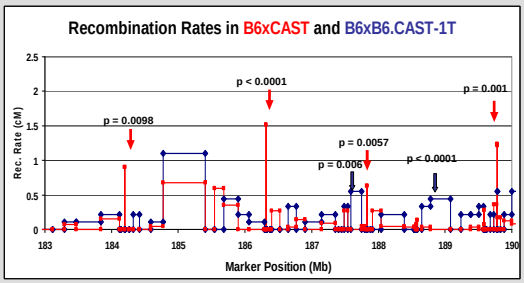

Fig.2 The recombination map shows suppression of recombination at specific hotspots and activation of other known hotspots coinciding with the absence of CAST alleles in the congenic cross. Red arrows – hotspots active in interstrain but disappearing in congenic cross; blue arrows – hotspots not present in the congenic cross.

Sites of crossover and non-crossover

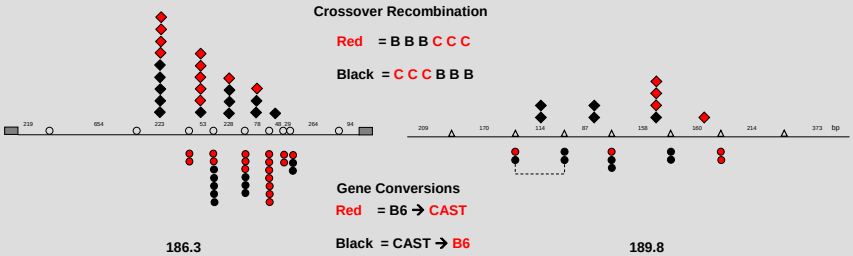

Fig.2 The sperm assay allows detection and discrimination between crossover and non-crossover events as well as their location. No direction bias (B->C or C->B) for both crossovers and conversion is observed and the conversion frequencies are equal to the crossover frequencies.

Early regulation of recombination by trans-acting factors

	Congenic Cross		Interstrain Cross	
Hotspot position	Gene Conversions	Crossovers	Gene Conversions	Crossovers
186.3	0	0	27	23
189.8	0	0	11	9

Table 1. The sperm assay confirmed the lack of crossover events at two known hotspots in congenic cross. In addition, the gene conversions were absent too, suggesting involvement of trans-acting genes in regulation of the early steps of recombination.

Conclusions:

The CAST alleles of trans-acting genes activate or suppress the activity of some hotspots.

The trans-acting factors must be involved in the early step of recombination, affecting both, the crossover and noncrossover events.

The hotspot phenotype segregates in F2 with possible involvement of more than one genes.

Mapping of trans-acting genes

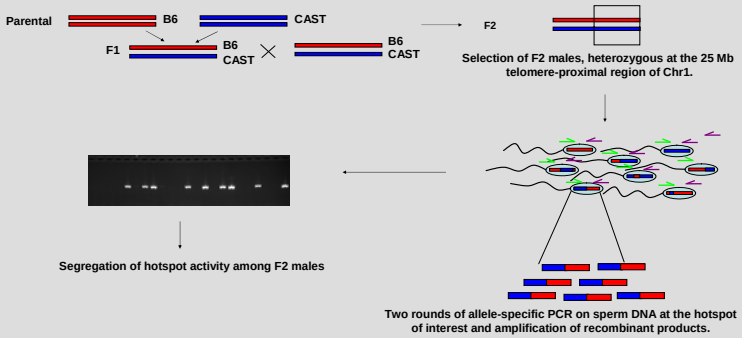

Fig. 3 Bulk sperm DNA assay of F2 males heterozygous at 25 Mb telomere-proximal region of chromosome 1.Two rounds of allele-specific PCR amplify the product of recombination at specific hotspot.

Segregation of hotspot activity among F2 males

Hotspot	Number of males with hotspot activity vs. total
186.3	20/40
189.8	19/40
191	20/40

Table 2. Number of individuals with detected activity of certain hotspots by allele specific PCR. The ratio between hotspot and nonhotspot individual is roughly 1:1 suggesting the role of at least one gene in regulation of the hotspot activity.

Numbers of F2 males with activity of different hotspots

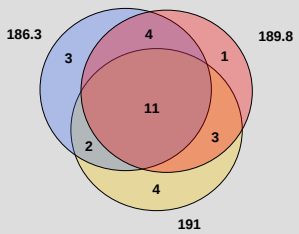

Fig. 4 The distribution of F2 males with one, two and three hotspots active propose the idea of presence of one single gene regulating the activity of all three hotspots, and other genes, specific for different hotspots.