



Trans-regulation of mouse meiotic recombination hotspots by *Rcr1*

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Summary

Meiotic homologous recombination generates genetic variety amongst offspring, and ensures that the chromosomes are segregated accurately during meiosis. Recombination occurs in hotspots, and the aim of this study was to identify *trans*-acting factors that control hotspot positioning in mammals, specifically inbred mice.

The study compared the location of crossovers in an 8-Mb segment of a 100-Mb region of mouse chromosome 1 where the longer region was heterozygous C57BL/6J (B6) x CAST/EiJ (CAST) and the remainder of the genome was either similarly heterozygous or entirely homozygous B6. This lack of CAST alleles had a major effect in hotspot activity, where several hotspots lost recombination activity, additional new hotspots were discovered, and some hotspots were unaffected. This suggested the impact of one or more *trans*-acting genes with CAST alleles that activated or suppressed hotspot activity.

Analysis of activity of three activated hotspots in sperm samples from males resulting from two genetic crosses identified a single *trans*-acting regulator of hotspot activity (*Rcr1*), on chromosome 17 and results from an *Escherichia coli* (*E. coli*) cloning assay suggested that *Rcr1* controlled the formation of the double-strand breaks that initiate the recombination process.

Introduction

Homologous recombination during meiosis is initiated by the formation of DNA double-strand breaks (DSBs) created by the highly conserved topoisomerase IV-like protein SPO11. These DSBs act as the sites for chiasmata and crossovers to form. The DSB repairs can either form a crossover between homologous chromatids (CO; exchange of genetic information) or a non-crossover (NCO; acquisition of genetic information by the initiating chromatid from its partner)^(2; 3).

In yeast and mammals the recombination rates vary along the chromosome^(4; 5; 6; 7; 8), and most occur in hotspots^(9; 10). Little is currently known about the causes behind the locations and activity of these recombination hotspots in mammals. *Trans*-acting factors controlling hotspot activation have been identified in several cases in yeast.

The aim of the study was to identify any *trans*-acting factors that control hotspot positioning in inbred mice. It focused on crossovers in an 8-Mb segment of a 100-Mb region of mouse chromosome 1, where the longer region was heterozygous C57BL/6J (B6) x CAST/EiJ (CAST) and the remainder of the genome was either similarly heterozygous or entirely homozygous B6.

Materials and methods

The mice

The mice were genetic crosses involving the B6 and CAST mouse strains. In the first cross (interstrain cross), B6 mice were mated to CAST, and the F1 hybrids were backcrossed to B6. In the second cross (congenic cross), B6 mice were mated to B6.CAST-1T, a congenic strain carrying 100 Mb of CAST DNA sequences from distal chromosome 1 introgressed into C57BL/6J.

The resulting F1 hybrids were then backcrossed to B6. The CAST alleles were present in the interstrain B6xCAST F1 animals and absent in the congenic B6xB6.CAST-1T F1 mice, which are homozygous B6 outside the 100-Mb chromosome 1 region.

E. coli cloning assay

The researchers applied the *E. coli* cloning assay to hotspots *Hlx1* and *Esrrg-1* as described in Ng et al.⁽¹¹⁾. The hotspot sequence was amplified with primers common to both fragments, and the cloning resulted in each colony representing a single DNA strand from the original meiotic event. An aliquot from each culture underwent fluorescent SNP genotyping.

Phenotyping

The assays used partially purified DNA as described in Paigen et al.⁽⁹⁾. To detect recombination activity, recombinant DNA fragments were selectively amplified at the hotspot sequence of interest.

Sperm DNA samples from 12-week old animals underwent two rounds of nested PCR using allele-specific primers in each of the two rounds. These were specific to the B6 alleles flanking the hotspot, and to the CAST alleles. The PCR conditions ensured that the product was only from recombinant and not parental DNA.

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Genotyping

The assays used partially purified DNA as described in Paigen et al. ⁽⁹⁾. Recombination activity in the region of 183.5–191.5 Mb on mouse chromosome 1 was fine-mapped using SNP markers.

For genome-wide association mapping (GWAS), the researchers genotyped all progeny at 20-Mb resolution using the KASP™ genotyping system, with markers selected from The Jackson Laboratory genotyping panel ⁽¹²⁾.

Fine mapping of CAST alleles on chromosome 17 used a combination of the microsatellite markers D17Mit48, D17Mit57, D17Mit113, and D17Mit46 and SNP markers.

Results / discussion

Trans-activation and suppression of hotspots

The B6xCAST recombination map was based on 6028 meioses in F1 animals. Among the F1 offspring, 735 had a single CO event in the 8-Mb region of interest (264 in females; 471 in males), which produced a sex-averaged map of 12.2 cM (8.8 cM in females; 15.6 cM in males).

The B6xB6.CAST-1T map was based on 2083 meioses, with 175 leading to single CO events (83 in females; 92 in males), which produced a sex-averaged map of 8.4 cM (7.1 cM in females; 10.1 cM in males).

The COs were mapped to hotspot-level resolution, and while most showed similar activity in the two crosses, six hotspots (*Fbxo28*, *Dusp10*, *Hlx1*, *D1Pas1*, *Esrrg-1*, and *Kcnk2*) disappeared in the congenic cross and three new and previously undetected hotspots appeared (*Capn2*, *Kctd3*, and *Ptpn14*).

There were statistically significant sex differences in three hotspots in the interstrain cross (*Hlx1*, *Esrrg-1*, and *Kcnk2*) and one in the congenic cross (*Kctd3*).

These results show that the recombination activity of a number of hotspots on chromosome 1 was affected (either activated or suppressed) by CAST allele(s) of distant *trans*-acting loci. Other hotspots were unaffected.

Mapping *Rcr1*, the *trans*-acting locus

The researchers used two mapping crosses to search for *trans*-acting genes regulating recombination at specific hotspots. The first was created by crossing the B6.CAST-1T congenic strain with CAST, and then crossing the F1 females with B6 (Figure 1A), creating mice that were heterozygous B6/CAST at the 100-Mb congenic region, with segregated CAST alleles in the rest of the genome. This allowed the detection of any relevant X-linked genes,

meant that all male progeny were informative; and that any CAST alleles were heterozygous.

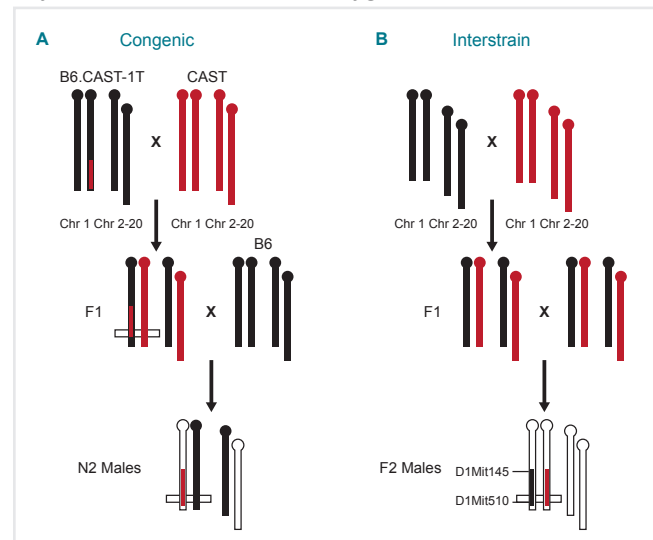


Figure 1. Crosses for Mapping of *Trans*-Acting Genes (B6.CAST-1TxCAST)xB6 (A) and B6xCAST (B) crosses used for mapping *trans*-acting genes. B6 sequences are in black, and CAST in red. The region where the recombination is studied is boxed. Segregating loci are located in the white regions. Note that any recombination within the congenic region between the B6.CAST-1T and CAST chromosomes does not alter the allelic composition of this region. doi:10.1371/journal.pbio.1000036.g003.

From this cross, 211 male animals were phenotyped for activity of the three hotspots *Hlx1*, *Esrrg-1* and *Kcnk2* using allele-specific sperm DNA assays. F2 animals from a second cross, mating B6xCAST F1 mice together, were also phenotyped.

Animals from both crosses were genotyped with 165 SNP markers, and this showed a strong link between hotspot activity and a locus located within a 5.30 Mb window on proximal chromosome 17. Both crosses produced identical map locations, and no other chromosome location showed significant linkage in either cross. This locus was designated Recombination regulator 1 (*Rcr1*).

The molecular identity of *Rcr1* is unknown, but the closest known phenotypic parallel is the *ADE6-M26* hotspot in *Saccharomyces pombe* activated via chromatin remodelling mediated by the *ATF1.PCR1* transcription factor.

Rcr1 controls the initiation steps of the recombination process

If *Rcr1* controls the early stage of recombination, it would control the appearance of both CO and NCO gene conversions at susceptible hotspots. If it acts on the choice

between CO and NCO, NCOs would persist at susceptible hotspots in the absence of the *Rcr1* CAST allele.

The researchers carried out a cloning assay counting the number of COs and NCOs at individual hotspots in F1 sperm DNA, and found that both NCOs and COs were absent from these hotspots, suggesting that *Rcr1*'s effect is at the initiation of recombination, before the choice between CO and NCO.

Conclusion

Trans-acting factors have potential to explain variations in hotspot activity, sex differences in the activities of individual hotspots, and the failure to find a consensus DNA sequence that accounts for the specificity of SPO11 cleavage.

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In a subsequent study⁽¹³⁾, the researchers were able to identify *Prdm9* as a mammalian protein regulating meiotic recombination hotspots which initiates studies of an important biological control system that has hitherto been inaccessible. The results of this successive study show that these sequences represent recombination hotspots. Additional studies in this and several other labs have shown that *Prdm9* controls activation of all, or nearly all, recombination hotspots.

Keywords: PCR, genotyping, fluorescence, allele-specific, recombination, meiosis, *trans*-regulation, chromosomes, crossovers.

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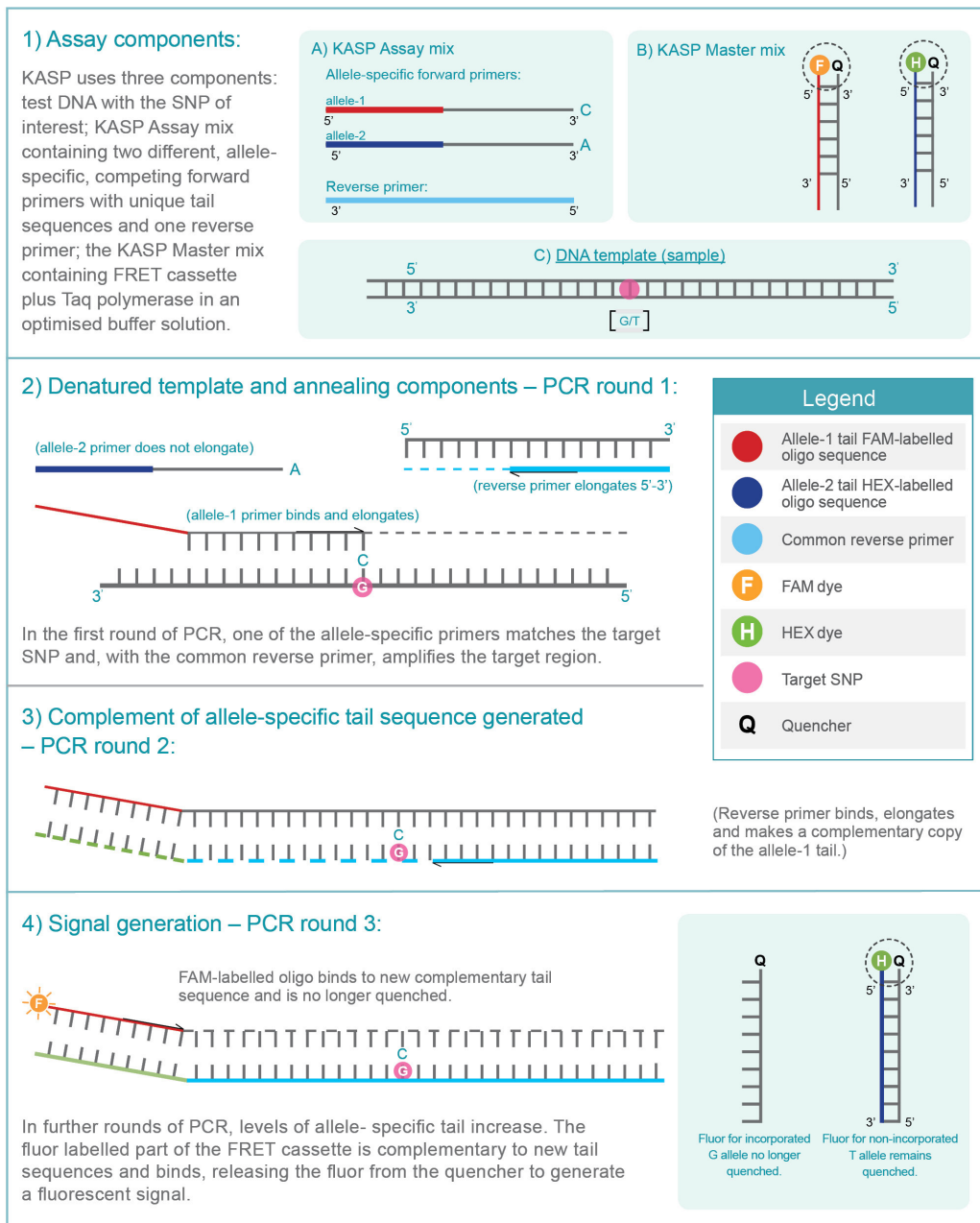
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