The trans-acting gene *Rcr1* regulates the placement and activity of recombination hotspots

Emil Parvanov, Petko Petkov, Siemon Ng, Kenneth Paigen

Center for Genome Dynamics, The Jackson Laboratory, Bar Harbor, ME, USA, emil.parvanov@jax.org

In mammals genetic recombination, which occurs at highly delimited sites along chromosomes, known as hotspots is required for both the completion of meiosis and production of genetic diversity in the offspring. Although the molecular events of the DNA exchanges are fairly well understood, very little is known about the factors regulating the location and relative activity of hotspots. We have now found a gene, *Rcr1,* on chromosome 17 whose CAST allele controls the activation of multiple hotspots on Chr1. *Rcr1* acts in trans to control the initiation of recombination as its CAST allele is required for the generation of both crossover and noncrossover gene conversion events.

Existence of trans-acting factors.

A comparison of the high-resolution recombination maps of telomere-proximal 8 Mb of Chr1 from two types of crosses showed that the absence of CAST alleles of trans-acting genes results in the loss of some hotspots and the appearance of new ones.

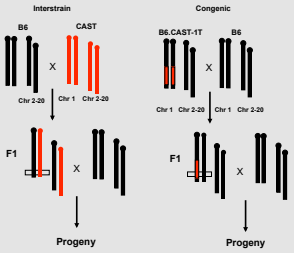


Fig.1. CAST alleles are present on all chromosomes in F1 of the interstrain cross. In congenic F1, CAST alleles are present only in the congenic region, the rest of the genome is homozygous C57BL/6J. Boxes indicate the region of recombination mapping.

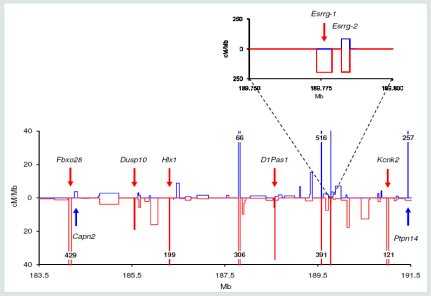


Fig.2 The recombination map after male meiosis shows suppression of recombination at specific hotspots and activation of other hotspots, coinciding with the absence of CAST alleles in the congenic cross. Red arrows – hotspots active in interstrain, but disappearing in congenic cross; blue arrows – hotspots present only in the congenic cross.

E.coli assay allows us to detect recombination events at hotspot areas and discriminate crossovers from gene conversions (Ng, S. H., E. Parvanov, et al. (2008). Genomics **92(4): 204-9**).

Sites of crossover and non-crossover

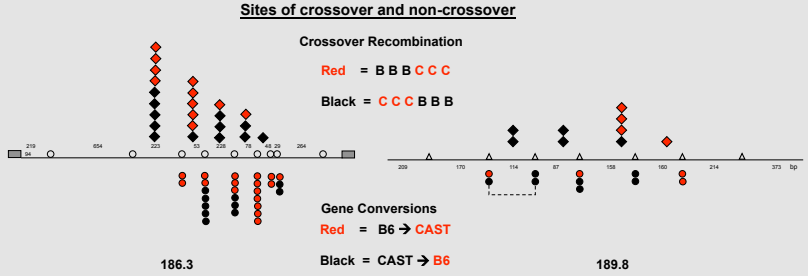


Fig.2 Sperm assays allowed detection and discrimination between crossover and non-crossover events as well as their location. No direction bias (B->C or C->B) for either crossovers or conversions was observed, and the conversion frequencies are equal to the crossover frequencies.

The trans-acting factors control initiation of recombination.

Both crossovers and gene conversions were lost in the absence of CAST alleles in the genome for two tested hotspots.

	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.

Mapping the trans-acting regulator *Rcr1*

PCR-based sperm phenotyping assay allows the detection of activity of selected hotspots in individual male animals in two mapping crosses.

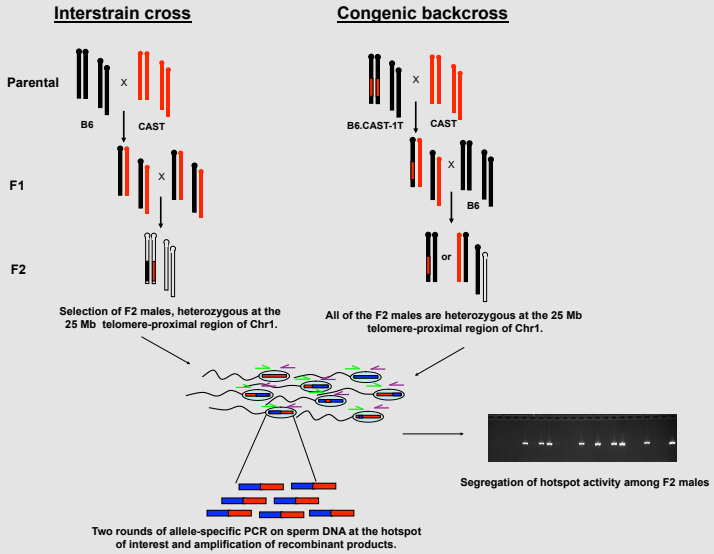
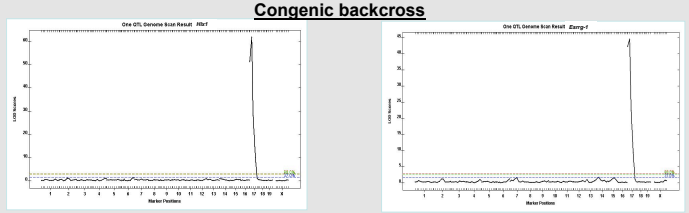


Fig. 3 Bulk sperm DNA assay of F2 males heterozygous at 25 Mb telomere-proximal region of chromosome 1.Two rounds of allele-specific PCR amplify the product of recombination at specific hotspot. More than 200 males tested from the congenic backcross and more than 100 males from the interstrain cross were tested.

A genome scan showed linkage between the activity of two hotspots on Chr1 and the CAST allele of a new locus, Recombination regulator 1 (*Rcr1*) at 12-17 Mb on Chr17

Congenic backcross



Interstrain cross

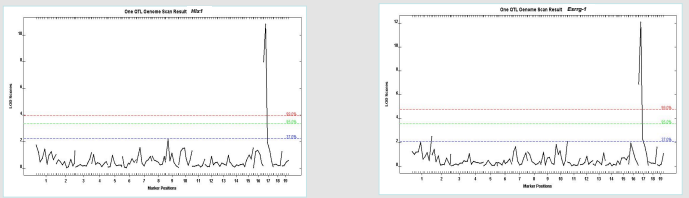


Fig.4 Data analysis by J/qtl software. Linkage between phenotype and genotype was observed in the centromere-proximal region on chromosome 17.