Distribution and control of recombination activities in mouse

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Abstract

Meiotic recombination creates genetic diversity by shuffling parental alleles while ensuring proper segregation of chromosomes during gamete formation. Using mouse as a model, we examined the following issues in recombination biology:

1. Recombination hotspot distribution and activity
2. Regional variations of recombination rate
3. Sex specificity of recombination
4. Effects of trans-acting factors controlling specific recombination hotspots

Mammalian recombination is known to occur at highly localized, 1-2 Kb regions called hotspots. We have now generated the first broad scale, high resolution map of hotspots along a mammalian chromosome, a 25 Mb region of mouse chromosome 1. Examining the distribution of hotspots and their frequencies showed that the majority of recombination events are concentrated in relatively few hotspots. These hotspots are flanked by cold regions between 5 to 600 Kb in length.

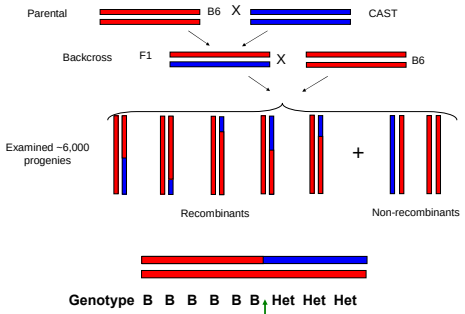
We have further detected regional and hotspot levels of sex specificity of recombination. On a regional scale, the telomere-proximal 10 Mb region on mouse chromosome 1 showed higher recombination rate in males than females whereas the more distal 10 Mb had lower rate in males. For individual locations, most hotspots are found in both males and females, but we have detected recombination activity biased towards one sex at some recombination sites.

Chromosomal differences in recombination rate were also observed in the mouse genome. Comparison between two 5 Mb regions of chromosomes 16 and 18 showed that the diminished activity in chromosome 16 is due to a reduction of overall hotspot intensities and not a decrease in the number of hotspots.

Using genetic crosses, we have found the first evidence that trans-acting factors control the activity of specific hotspots. These control factors are specific to individual hotspots and can either activate or suppress recombination activity. Fine mapping two hotspot loci using a new cloning assay demonstrated that the trans-acting factors affect both cross-over and conversion recombination, suggesting that their primary action is on the initiating events of recombination.

Mapping Recombination Hotspots

We examined recombination hotspots in 6,000 mice generated from F1 (C57BL/6J x CAST/EiJ) backcrossed to C57BL/6J.

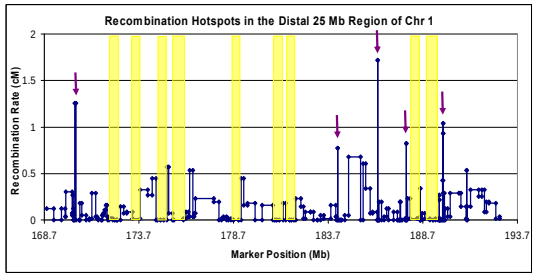


Recombination events are detected by SNP genotyping. A transition from homozygous (B6-B6) to heterozygous (B6-CAST) genotype would indicate a cross-over had occur between two SNPs.

Recombination events are limited to relatively few hotspots

A 25 Mb region of chromosome 1 was mapped at 200Kb resolution:

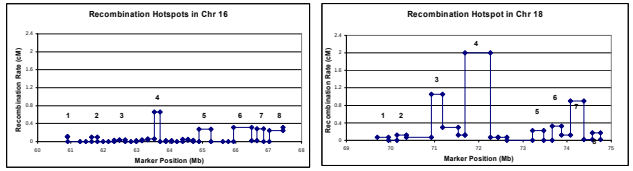
1) Hotspots are surrounded by cold spots upwards of 600 Kb. ( )
2) Activity of hotspots can vary by 100 fold (Highly active hotspots ↓).
3) 50% of all detected events are localized to a few highly active hotspots



Hotspot activities are influenced by regional variation

Compared activity of hotspots on chromosome 18 and chromosome 16:

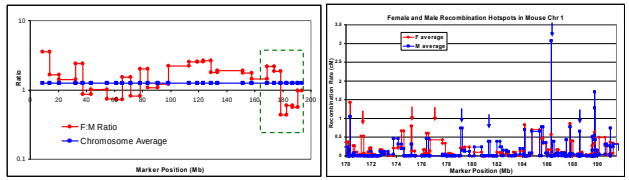
1) Two regions have similar number of hotspots
2) Hotspots on Chr 16 are less active than hotspots on Chr 18



Sex influences hotspots at both local and regional levels

Mapped reciprocal F1 backcross (♀F1x♂B6 or ♂F1x♀B6):

1) Telomeric region shows higher recombination activity in males than females.
2) Some hotspot are biased towards one sex.



Conclusions

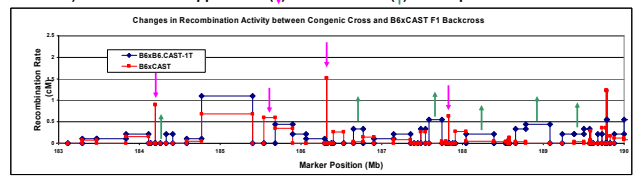
Using mouse as a model for mammalian meiotic recombination, we found:

1. Recombination hotspots are flanked by long cold spots in the mouse genome
2. Recombination activities are influenced based on chromosomal location
3. Some hotspots are active in only one sex
4. Trans-acting factors alter both cross-over and conversion activity

Discovered the first evidence for trans-acting factors

Mapped hotspots in chromosome 1 of a B6.CAST-1T congenic:

1) Detected both suppression (↓) and activation (↑) of hotspots

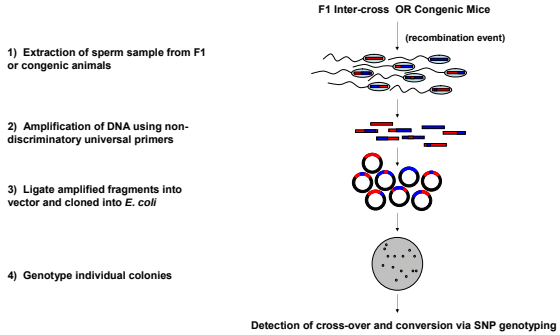


We mapped a congenic mice strain where the distal region of chromosome 1 is replaced by CAST specific alleles in a B6 background. Changes in recombination hotspots between the congenic and the F1 inter-strain can be directly attributed to the lack of CAST alleles located elsewhere in the genome.

Trans-acting factor affects both recombination events

LOSS OF BOTH CROSS-OVER AND CONVERSION IN THE CONGENIC CROSS.

1) Trans-acting factor influences the recombination pathway early in the process, possibly in determining the location of the initial double strand break.
2) Developed a new *E. coli* cloning assay for sperm genotyping



Each individual *E. coli* colony represents an individual sperm. This newly developed assay allows for single sperm genotyping of multiple SNPs.

Two hotspot loci located at 186.3 Mb and 189.8 Mb showed loss of cross-over and conversion in the congenic cross.