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RESEARCH HIGHLIGHT

Meiotic DSBs and the control of mammalian recombination

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The laboratories of Galina Petukhova and R Daniel Camerini-Otero have achieved significant technical advances in determining the genome-wide sites of DNA double-strand breaks (DSBs) where the process of genetic exchange between chromatids during meiosis begins. Applying the new approaches to male meiosis in mice, their experimental results considerably increase our insights into the nature and regulation of these processes.

Genetic recombination is essential for successful meiosis in all sexually reproducing organisms, where it plays the dual roles of assuring proper segregation of chromosomes in Meiosis I and generating new allelic combinations that serve as the substrates for evolutionary selection. The importance of recombination in this latter role is enhanced by the fact that there is a rapid evolution of new genes arising by tandem duplications of existing genes whose new copies are now available for altered or even entirely new functions [1], but this close proximity requires that forming new combinations of these functions depends on genetic recombination among closely linked genes. In a more empirical context, genetic recombination plays the operational role of making the science of genetics possible; it is how we determine the relative positions of genes along chromosomes, map the locations of novel phenotypic determinants, and analyze the genetic structures of chromosomal rearrangements.

In 1982 Steinmetz and Hood observed that genetic recombination in the major histocompatibility region of the mouse genome did not occur randomly along the DNA sequence, but instead was concentrated in short regions that were separated by much longer regions devoid of recombination [2]. They named these regions of high recombination hotspots, and the name has stuck ever since. Over the succeeding years we learned that hotspots, as well as being present in mice, are found in humans, dogs, yeast, and *Arabidopsis*, but curiously are thought not to be present in *C. elegans* or *Drosophila* species, although definitive, high-resolution molecular studies have yet to be carried out in these latter exceptions.

The recent advent of SNP genotyping in mammals made it possible to construct genetic maps of entire chromosomes at kilobase-level resolution and map the locations of recombination hotspots with very high resolution. It was then possible to characterize their properties in a systematic way and to address the critical questions of why they are where they are; what determines their relative activities as sites of genetic recombination (something that can vary over several orders of magnitude), and why they sometimes differ between the sexes. In humans, this was achieved by finding that recombination hotspots define the boundaries of blocks of linkage disequilibrium (series of adjacent SNPs that have failed to undergo recombination over many generations because they lack internal hotspots) [3]. In mice, systematic hotspot identification required mapping thousands of SNPs among thousands of progeny from crosses between inbred strains [4]. Studies in both species identified a zinc finger protein with histone H3K4 methyltransferase activity, PRDM9, as a critical factor determining hotspot locations in mammals [5-7]. PRDM9 had previously been described by Hayashi *et al*. [8] as an essential meiosis-specific transcript, probably involved in the control of meiotic transcription, with a knockout phenotype of arrest before the first meiotic division. The current model posits that PRDM9 binds to hotspot recognition sites in DNA and trimethylates nearby histones, thereby activating chromatin and attracting the topoisomerase SPO11, which then catalyzes the initial DNA double-strand break (DSB) that physically begins the exchange of DNA sequences between recombining chromatids.

Now, the laboratories of Petukhova and Camerini-Otero have jointly provided a series of papers that introduce a dramatically new capability in studying hotspot functions, one that has already provided intriguing new results and promises to open up new possibilities in the study of genetic recombination mechanisms [9-11]. Their methodology exploits the single-stranded (ss) DNA tails that arise on either side of the newly formed DSBs. The proteins DMC1 and RAD51 associate with these

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ssDNA tails, making it possible to determine the bound DNA sequences by ChIP using antibodies directed against these proteins. The methodology has been further improved by introducing a DNA isolation protocol that greatly enriches for ssDNA and removes nearly all dsDNA background. When combined with a new computational framework, the result is an exceptional ability to describe the location and relative activity of hotspots of DSB formation.

Using this approach, they have described the locations of DSB hotspots in congenic strains of C57BL/10 mice that are almost identical genetically except for carrying either the 9R or 13R alleles of *Prdm9*, as well as the F1 hybrid between them. They also tested C57BL6J (B6) mice and B6 mice carrying a knockout of *Prdm9*. When present as homozygotes, the 9R and 13R *Prdm9* alleles could each activate ~15 000 hotspots, with very little overlap between them. This is the strongest indication yet that nearly all hotspots are PRDM9-dependent. Each allele-specific set of hotspots was characterized by a distinct DNA motif at the center of the hotspots, the extent of whose match to the PRDM9 consensus sequence correlated with hotspot strength. Confirming the role of trimethylation in hotspot activation, they found that nearly all DSB hotspots coincided with allele-specific H3K4me3 marks (determined by ChIP), and importantly, that these marks also formed in *Spo11*-null mice, confirming that they precede rather than follow DSB formation.

It appears that PRDM9 not only acts as a homing signal for SPO11, but is also required to prevent ectopic DSB formation. Tested in B6 mice, the 9R allele activated slightly more hotspots (~18 000), and the *Prdm9* knockout activated a comparable number, but at new sites. Very importantly, the majority of these new hotspots were located near transcription start sites, where histone H3K4 is also trimethylated, most strongly at the +1 nucleosome. They concluded that SPO11 action is restricted to hotspots when PRDM9 is present, but that SPO11 defaults to other sites of trimethylation in its absence.

Exceptionally, PRDM9 does not activate the obligatory genetic crossover in male meiosis that occurs at the boundary of the pseudo-autosomal region (PAR), the region that is shared by the X and Y chromosomes. This obligatory recombination event arises in a tight cluster of hotspots that, unlike the rest of the genome, show no preference for *Prdm9* alleles and remain active in *Prdm9*-null mice, indicating that the PAR region-independent hotspots are activated by an alternative mechanism. Regional aspects of chromatin structure play a role in this unusual control as PRDM9-independent and -dependent hotspots are intermixed in a gradient nearly 900 Kb long adjacent to the PAR. Support for the existence of an alternative mechanism comes from the observation that dogs, which lack a functional *Prdm9* allele [12, 13], nevertheless have recombination hotspots [13]. The existence of this alternative is driven by the need to overcome the paucity of DSBs relative to hotspots. There are about 200-400 DSBs formed in a meiotic cell; these are distributed among the 15 000 or so potential sites on each of four chromatids, or about 1 in 200 possibilities. Overcoming these odds and assuring recombination at the PAR apparently requires an additional mechanism.

Nearly all of the 15 000 hotspots present in F1 hybrid mice were present in one of the parents. That the number of F1 hotspots is not the sum of those present in the two parents makes it clear that there is a regulatory cap on the number of hotspots allowed. Within this cap there is a distinct competition among hotspots; the winners appear to be drawn from the most active hotspots activated by each allele, with the 13R allele providing 75% and the 9R only 25%. This disproportion indicates that *Prdm9* alleles are not equally powerful; this difference could result from allelic differences in levels of expression or from their relative affinities for their DNA-binding sites. To the extent this latter possibility is important it may well reflect the relative age of each allele. Boulton *et al*. [14] pointed out a number of years ago that because the chromatid on which the initiating DSB occurs is repaired using its intact partner as a template, mutations within hotspots that reduce their activity should be selected over time until an entire family of hotspots gradually fades away. After considerable discussion in the literature, this so-called "hotspot paradox" was resolved with the discovery of PRDM9 and the realization that entirely new families of hotspots could be created at once by mutations in the DNA-binding zinc finger domain of PRDM9, and indeed, the DNA-binding contact amino acids in the zinc fingers are under intense evolutionary selection [15, 16].

It is now clear from the work of the Petukhova and Camerini-Otero labs that with the exception of the PAR, virtually all DSBs occur at PRDM9-marked hotspots; that there are stronger and weaker alleles of *Prdm9*, each with characteristic DNA-binding motifs; that trimethylation of histone H3K4 by PRDM9 is a requirement for localizing DSB formation and not a consequence; and that the functions of PRDM9 extend beyond marking hotspot sites to preventing ectopic DSBs and assuring that only PRDM9-marked sites suffer DSBs.

One beauty of the work described here is that beyond clarifying multiple important aspects of genetic recombination, their results generate new questions as well as emphasizing the old questions that still require answers: what limits the average number of DSB per meiocyte to ~300 despite the presence of an excess of SPO11; what makes some *Prdm9* alleles stronger than others; what regulates the frequency with which DSBs are resolved as crossover vs non-crossover gene conversions and

why is this sometimes sex-specific; what accounts for PRDM9-independent recombination; and how can our newly enhanced understanding of the functions of PRDM9 inform its presently enigmatic role in determining hybrid sterility between sub-species of mice [17].

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