sity of Northern Hemisphere summer sunlight (insolation), the latter calculated from known changes in the geometry of Earth's orbit and rotation axis. This observation provided support for the Milankovitch (*6*) or astronomical theory of the ice age cycles. However, the early data also posed a serious problem. Calculated sunlight variations have periods of ~23,000, ~41,000, and ~100,000 years, with the latter periodicity much weaker than the others. The ice age cycles contain the same periodicities, but for the last several cycles, the dominant period is ~100,000 years, the least significant in the sunlight calculation (*4*). A generation of scientists has strived to explain this "100,000-year problem" through nonlinear responses of Earth's climate to changes in the seasonal distribution of sunlight (*7*, *8*) or to processes not related to the sunlight calculation at all.

Dorale *et al*. provide evidence for high sea level at ~81,000 years ago, in the middle of the most recent 100,000-year cycle. This result challenges the observational basis for much of the discussion over recent decades. A high sea level at ~81,000 years ago is not consistent with a 100,000-year beat, but it does coincide with calculated high Northern Hemisphere summer sunlight, and thus supports a simple version of the Milankovitch theory. If verified, this sea-level high may be considered an exception to the 100,000-year cycle, in which high summer sunlight caused the ice sheets to melt—an exception with precedent, given evidence for another off-beat event ~229,000 years ago (*7*).

Dorale *et al*. dated layers of the mineral calcite, which were deposited like bathtub rings from pools of water in Mallorca caves, in the western Mediterranean. Because the pools are connected to the sea through underground passages, the layers record sea level at the time they formed. Using this approach, Dorale *et al*. inferred sea levels similar to modern values ~81,000 years ago. They estimated maximum rates of sea level rise of ~2 m per century. This rate is high, but not unprecedented in the geologic record. It exceeds by several times those predicted for the next century (*9*).

Others will likely test Dorale *et al*.'s inference of low ice volume 81,000 years ago. A major question relates to the flow and bend of the solid Earth, such that sea level is not solely dependent on ice volume. Earth's shape, mass distribution, and gravitational field change continually in response to the redistribution of water between the ice sheets and oceans during the ice age cycles (*10*, *11*). Because of the high viscosity of Earth's mantle, the solid Earth responds slowly (over thousands of years) to the rapid redistribution of water and ice on the surface. The physics of this process is well known, but the calculation requires knowledge of the elastic properties of the lithosphere (Earth's rigid outer shell), the viscosity structure of the mantle and the history of ice and water distribution on Earth's surface, which are difficult to quantify. Nevertheless, the process has been modeled for different times in the ice age cycle (*10*, *11*). Dorale *et al*.'s findings should spur further studies, with an eye toward the Mallorca region.

A number of previous studies have estimated sea level ~81,000 years ago. Some of these estimates appear to agree (*12*) with Dorale *et al*.'s findings, whereas others appear to disagree (*13*). One problem with comparing these studies is the possibility—and in some cases probability—that discrepant sea-level elevations may represent different sea levels at different times, given plausible dating errors. Future studies that determine sea levels at different times at the same place may help to resolve the discrepancies. Regardless of the ultimate verdict on sea level ~81,000 years ago, Dorale *et al*.'s findings will stimulate ideas, discussion, and new studies of ice age history and causes.


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GENETICS

# Genetic Control of Hotspots

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Both chromatin and DNA sequence account for individual differences in the location and frequency of genetic recombination.

With the exception of identical twins, individuals have different genetic makeup, which results from two key processes. During meiosis, maternal and paternal homologous chromosomes assort randomly to form daughter cells (gametes), thus generating different combinations of maternal and paternal chromosomes. Additional variation is generated by recombinations or crossovers, in which parts of homologous chromosomes are exchanged, resulting in a new combination of parental alleles. On pages 835, 836, and 876 of this issue, Parvanov *et al*. (*1*), Baudat *et al*. (*2*), and Myers *et al*. (*3*) report the identification of a mammalian gene—PR domain containing 9 (*PRDM9*)—that controls the extent to which crossovers occur in preferred chromosomal locations, known as "hotspots" (see the figure).

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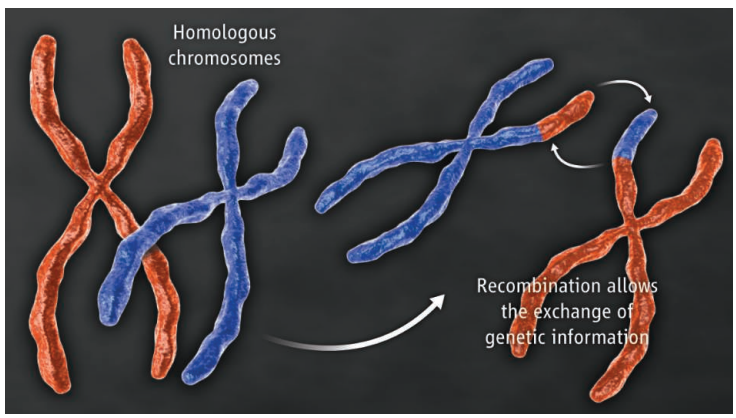
In addition to contributing to genetic variation, recombination is critical to the success of meiosis. A physical bridge that is built around the point of exchange—the chiasma—ensures correct assortment of chromosomes into gametes. The location of a chiasma is important because an exchange that occurs too close to the telomere or centromere of a chromosome can confer instability and lead to abnormal chromosome segregation (*4*). In humans, this type of error is startlingly common. Aneuploidy [either monosomy (only a single copy of a chromosome, rather than a pair) or trisomy (three copies of a chromosome)] is estimated to occur in 10 to 25% of all conceptions and is the leading cause of pregnancy loss as well as developmental disabilities (*5*).

Even though meiosis and meiotic recombination are fundamental cellular processes, the genes and mechanisms involved are poorly understood. In mouse recombination hotspots, histone proteins are often modified by methylation or hyperacetylation (*6*),

and in many human hotspots, a degenerate DNA sequence of 13 nucleotides (a 13-mer) is found (*7*). These findings have generated debate over whether individual variability in recombination rates and locations of crossovers is explained by DNA sequence signatures or epigenetic marks.

PRDM9 is a zinc finger protein that acts as a histone methyltransferase that trimethylates lysine 4 of histone 3 (H3K4me3). The *PRDM9* gene is highly polymorphic, especially in its zinc finger arrays that bind DNA. The polymorphic forms of PRDM9 recognize different DNA sequences and therefore can promote crossovers at different chromosomal sites among individuals. The finding that a polymorphic chromatin modifier binds to different DNA sequences is pivotal, because it suggests that both chromatin and DNA sequences are important in meiotic recombination.




**Genetic variation by crossover.** Recombination events (crossovers) occur at different sites along chromosomes. Some sites, so-called "hotspots," are used more often than other sites.

One elegant feature of the three studies is that *PRDM9* was identified through mouse and human studies and by computational analyses. The mouse and human work treated the numbers of crossovers as quantitative phenotypes in genetic analyses. In the mice, linkage scans were used to identify the chromosomal regions that segregate with high and low recombination rates in known hotspots. Parvanov *et al*. used a mouse cross to fine-map the candidate regions to four genes, and argued that of these, *PRDM9* is the only logical candidate gene. Similarly, Baudat *et al*. narrowed a region that they mapped previously (*8*) and identified *PRDM9* as the candidate gene. They then took the study directly into a human population of Hutterites and found three allelic forms of *PRDM9*: A, B, and I on human chromosome 5. The A form was the most common and accounted for 94% of alleles. They further noted that the I allele encodes a protein that does not recognize the 13-mer hotspot motif. The authors show that usage of hotspots by heterozygous individuals who carry one copy of A and a copy of I or B forms of PRDM9 is significantly different from that of individuals with two copies of the A form of PRDM9. The variation in the zinc finger array alone accounts for 18% of phenotypic variation. Myers *et al*. used a computational approach and found that human PRDM9 is the only zinc finger protein that binds to the 13-mer motif. They also demonstrated that the 13-mer motif does not appear to be active in chimpanzees. By cross-species comparisons, they suggest that the difference may be attributed to rapid evolution in humans rather than loss in chimpanzees.

An important lesson from this discovery is that results from genetic studies can direct mechanistic analyses. Often, variation is an unwanted complication in mechanistic studies; here the authors took advantage of the variation in recombination events to identify *PRDM9* as the genetic determinant and paved the way for examining the role of chromatin in recombination. Analyses of yeast and mouse hotspots have shown that H3K4me3 markers are enriched in recombination initiation sites (*6*, *9*). Results from Myers *et al*., Baudat *et al*., and Parvanov *et al*. further support the role of chromatin modifiers. Despite using fairly crude phenotypes (for humans, the phenotype was the percentage of recombinations in hotspots), these investigators were able to identify a single gene, thus suggesting that variation in recombination might be regulated by a few genes with large effects. Identifying additional genes will refine future mechanistic studies.

What are the interacting partners of PRDM9 and how do they work together to direct recombination? Not all the sites in the human genome with the 13-mer motif have a crossover, so it is unclear how PRDM9 decides which sites to use. Additionally, because *PRDM9* explains only an estimated 18% of individual variation in hotspot usage, other genes must contribute to this variation. If more fine-scale phenotypes such as specific types (e.g., those with the 13-mer motif) of recombination are used, it may be possible to remove some measurement noise and therefore have greater power to detect genetic association. Previously, other human genes including *RNF212* were identified as genetic determinants of the number of recombinations per meiosis (*10*, *11*); perhaps there are interactions between these genes and *PRDM9*.

At the interface between mechanisms and disease, an important question is whether there is a minimum number of recombinations required for proper meiosis. In yeast, when crossover events were diminished by deletion of the histone methyltransferase Set1, new crossovers appeared in trimethylation "deserts" (*9*). This and other studies (*12*, *13*) suggest a minimum number of required recombinations for proper meiosis; if so, what genes or pathways monitor this process? If this global monitoring system (which likely includes PRDM9) fails, aneuploidy may result. If this is the case, an enrichment of certain *PRDM9* variants would be expected among women with recurrent miscarriages and infertility and those who have had aneuploid pregnancies. The finding of the three studies opens the door to understanding the balance of successful gamete formation and maintenance of genetic diversity. Ultimately these studies will have clinical impact beyond basic science, as they will inform us about how errors of recombination lead to infertility, miscarriages, and developmental disabilities.

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