

Setting the Stage for Chromosome Breakage

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Homologous recombination is a means by which a broken chromosome uses its intact sister chromatid or homologous chromosome (homolog) as a template for repair. In the majority of sexually reproducing organisms, recombination during meiosis is critical to facilitate pairing of homologous chromosomes and subsequent segregation of homologs (Petronczki et al., 2003). Crossovers also contribute to increased genetic diversity.

Meiotic recombination is initiated by programmed DNA double-strand breaks (DSBs), which generally occur in regions termed hotspots. How these hotspots are specified has been unclear. Crossovers also happen outside of hotspots, however, and how this occurs is also unknown. To address these outstanding issues, research scientist Kyle Fowler and colleagues in the laboratory of Dr. Gerald Smith (Basic Sciences Division) and collaborators at Memorial Sloan Kettering Cancer Center used a high-sensitivity assay for measuring DSBs in meiotic cells of the fission yeast *Schizosaccharomyces pombe*. "We found, much to our delight, the solution to a paradox we had had for nearly fifteen years in our lab – how crossovers between homologous chromosomes arise in regions in which we could not detect DNA breaks. Because the background of this new assay is so low, we could see breaks in places they had not been detected before," said Dr. Smith.

To determine sites of breakage across the genome, the researchers took advantage of the fact that the Rec12 protein, which initiates DSB formation during meiosis, remains bound to a 13-29 base pair oligonucleotide excised from the genome. They immunoprecipitated Rec12 and subsequently purified the associated oligos, which were then sequenced. The sequenced reads were then used to generate a genome-wide map of DSBs. The sequencing-based map was in good agreement with maps of DSBs previously generated by Rec12 chromatin immunoprecipitation (ChIP) and Southern blots, and DSBs were generally not detected in regions without detectable recombination.

Strikingly, many DSBs were found outside of hotspots. While these "cold regions" displayed a relatively low mean density of Rec12 oligos, their mean Rec12 oligo density was ~32-fold higher than that at the recombinationally suppressed rDNA, arguing that these cold region DSBs are legitimate. Furthermore, the oligos arising from both hotspots and cold regions displayed similar sequence compositions, arguing that breaks in both classes of regions arise by similar

mechanisms. Notably, the authors found that DSBs in cold regions were about four times as likely as those in hotspots to give rise to crossovers, further indicating the importance of these non-hotspot DSBs to recombination.

Chromatin structure influences DSB formation in many organisms, and so the authors made use of data generated previously by micrococcal nuclease (MNase) digestion of chromatin in meiotic *S. pombe* cells. MNase cleaves accessible DNA and "nibbles" DNA until it reaches an obstruction, such as a nucleosome. This analysis revealed that DSB hotspots were not associated with strong nucleosome depleted regions (NDRs), in contrast to what has been seen in the budding yeast *Saccharomyces cerevisiae*, though there was weak nucleosome depletion at hotspots relative to flanking regions.

In *S. pombe*, certain transcription factors (TFs) are known to influence DSB formation. Analysis of the overlap between hotspots and sites bound by Atf1-Pcr1 TF revealed a relatively small number of overlapping sites. Extending this analysis, the authors also analyzed concordance between hotspots and sites predicted to be bound by the Rst2, and Php2-Php3-Php3 TFs, which again revealed a relatively small degree of overlap. Together, these results argue that the contribution of these TFs to the overall landscape of DSBs is relatively small.

Lastly, the authors analyzed the distribution of Rec12 oligos around all 4,096 possible 6 base pair DNA sequences. They found an association between Rec12 oligos and two specific sequences, ACACAC and ACTGCT. These sequences were especially sensitive to MNase digestion, suggesting that they lie within accessible chromatin that would facilitate DNA breakage.

The results presented in this study shed light onto both chromosomal determinants of DNA breakage and the role of non-hotspot DSBs in recombination. "Putting our results together with other observations in our lab, we found that breaks in these cold regions are about four times more likely to give a crossover than are breaks in "hotspots," small chromosomal regions where breaks are up to two hundred times more likely to arise than in cold regions. Our data show that 'crossover invariance,' the nearly uniform distribution of crossovers in spite of break hotspots, extends across the whole genome. Earlier data from our lab showed that hotspot breaks are repaired primarily with the genetically identical sister chromatid and cannot give a crossover, whereas cold-region breaks appeared to be repaired with the homolog and can give a crossover. We are now facing the problem of how this differential repair occurs," said Dr. Smith.

[Fowler KR, Sasaki M, Milman N, Keeney S, Smith GR](#). 2014. Evolutionarily diverse determinants of meiotic DNA break and recombination landscapes across the genome. *Genome Res* Epub ahead of print.

See also: [Petronczki M, Siomos MF, Nasmyth K](#). 2003. Un menage a quatre: the molecular biology of chromosome segregation in meiosis. *Cell* 112(4):423–440.

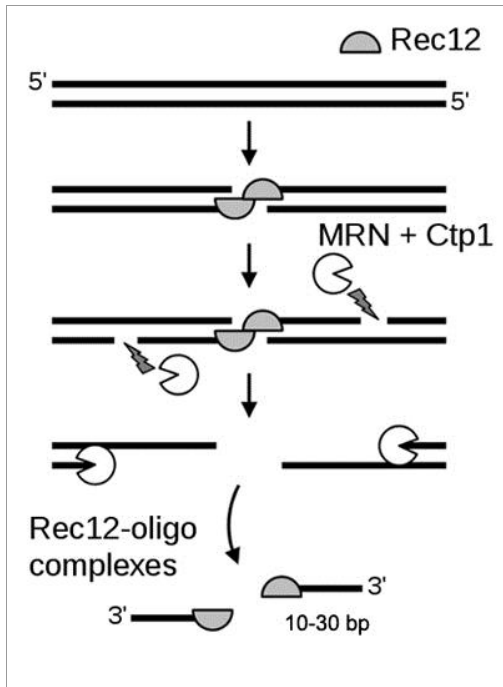


Image provide by Dr. Gerald Smith

The pathway by which meiotic double-strand breaks (DSBs) and Rec12-oligo complexes are formed. Two Rec12 molecules bind to and cleave duplex DNA, after which the Rec12 molecules remain bound to the DSB 5' ends. Subsequent endonucleolytic cleavage by the MRN (Mre11-Rad50-Nbs1) complex and Ctp1 releases Rec12 with an attached short oligonucleotide.