RECtify Meiotic Recombination

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The ultimate goal of meiosis, the type of cell division that occurs in gametes, is to produce haploid cells with genetic diversity. This is somewhat paradoxical since a form of genetic instability (recombination) must be invoked to generate diversity but overall genetic stability has to be maintained to guarantee viability. Meiosis uses homologous recombination, an exchange of genetic material between homologous chromosomes, for this purpose. DNA double-strand breaks (DSBs) are required for homologous recombination, but it has remained unclear how the DSB machinery is activated. The Smith Lab in the Basic Sciences Division has made pivotal advancements toward understanding the protein machinery required for meiosis by studying the process in the fission yeast *S. pombe*.

In this organism and others, regions of the genome called “hot spots” are preferred sites of DSB formation. Although certain transcription factors and histone modifying enzymes have been implicated in hot spot specification, none of them are absolutely required for determining hot spots genome-wide. Therefore, Fowler *et. al.* decided to study the protein determinants of hot spot formation at a global level to determine factors that promote DSBs at specific genomic loci. According to Dr. Smith, “people have looked for proteins that determine meiotic DSB hotspots for 20 years or more. Finding these, the first that act across the genome, will help us work out how the genome sites for DSBs are chosen, what might drive their evolution, and how they might be controlled to allow hotspots at will."

S. pombe has structures called linear elements (LinEs) that are similar to the synaptonemal complexes in other organisms that physically pair homologous chromosomes together to promote homologous recombination. As a starting point, the researchers analyzed the localization of GFP-tagged LinE proteins Rec10, Rec25, Rec27, and the newly identified Mug20 to meiotic chromosomes. They found that the proteins were interdependent for chromosome localization and localized to chromosomes independent of DSB formation. To obtain high resolution mapping of the genomic binding sites of the LinE proteins, ChIP-chip was performed on the GFP-tagged proteins. The authors were surprised to find a high enrichment of LinE proteins to DSB hot spots (up to 80 times the genome median at some hot spots) but not in DSB cold regions. Moreover, the relative amount of protein bound at a particular hot spot corresponded with the level of DSB formation, suggesting that protein binding somehow stimulates the DSB machinery.

If LinE proteins contribute to hot spot DSB formation, their deletion should decrease the amount of DSBs produced. Indeed, *rec27*mutants failed to generate DSBs at 80% of the hot spots and *rec10* mutants had almost no DSB formation. Fowler *et. al.*revealed that Rec27 does not require DSB formation to localize to hot spots since Rec27 localization in DSB formation-deficient*rec12* cells was similar to wild-type (see figure). Also, the researchers showed that the loss of proper Rec27 localization in *rec8*cells, a cohesin mutant that fails to localize all LinE proteins, decreased hot spot DSBs. Altogether, these data reveal a key role of Rec27 and other LinE proteins in hot spot functionality.

To further explore the protein landscape at hot spots, the authors analyzed the genome-wide localization of Rec12, the enzyme that directly produces the DSBs. They found that Rec12 has a modest preference for DSB hot spots but also binds to other genomic regions. Moreover, the mere presence of Rec12 did not always correspond to DSB production, indicating that Rec12 must be activated at hot spots to generate the DSBs. Fowler *et. al.* found that hot spots where both Rec27 and Rec12 co-localized had DSB formation, indicating that Rec27 or an interacting protein facilitates Rec12 activity at hot spots.

The body of work presented in this new manuscript from the Smith Lab reveals that *S. pombe* LinE proteins play a critical role in DSB formation at hot spots. These proteins are enriched nearly exclusively at hot spots and they are the best predictors for hot spot position in any species reported to date. An exciting future endeavor will be to determine the mechanism by which Rec27 and other LinE proteins activate Rec12 to generate the DSBs that are necessary for gametogenesis, a process that propagates and evolves life.

Fowler KR, Gutiérrez-Velasco S, Martín-Castellans C, Smith GR. 2013. Protein determinants of meiotic DNA break hot spots. *Mol Cell*. 49(5):983-96.

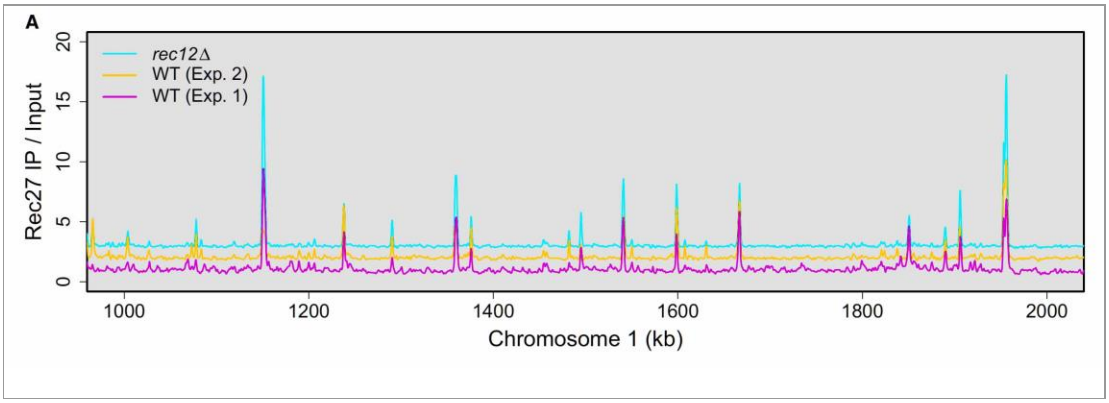

Figure adapted from the manuscript

Rec27 binding to hot spots is upstream of DNA double-strand break (DSB) formation. ChIP-chip data reveal that the localization of Rec27 to genomic hot spots (distinct peaks correspond to Rec27 enrichment in regions of Chromosome 1) is unperturbed in the absence of the DSB enzyme Rec12 (blue line). Thus, Rec27 binding to hot spots precedes DSB formation and the authors speculate that Rec27 promotes DSB generation by a currently elusive mechanism.