Insights into Holliday Junction Resolution and Chromosome Segregation in Yeast

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Homologous DNA recombination is the highly conserved process by which similar or identical nucleotide sequences are exchanged between two DNA molecules. In all eukaryotes – from yeast to humans – this kind of genetic recombination serves two critical functions during meiosis, the class of cell division that leads to the formation of gametes such as sperm and eggs in the case of humans, or spores in yeast: Homologous recombination promotes the genetic diversity of gametes by allowing crossing-over between homologous chromosomes, and it is also critical to accurate chromosome segregation during meiosis. Anomalies in recombination often result in the complete loss of chromosomes during meiosis, or in chromosomal rearrangements such as deletions or translocations, which in turn can lead to birth defects or cancer.

All current models for crossing-over involve two key intermediate structures: DNA double strand breaks (DSBs) and Holliday junctions (HJs). DSBs are the initiating events in crossing-over that are caused by Rec12 in fission yeast, whereas HJs are the crossed-strand structures by which DNA molecules from two homologous chromosomes become intertwined (see figure). At the Fred Hutchinson Cancer Research Center, the laboratory of Dr. Gerald Smith (Basic Sciences Division) has made many advances toward understanding a complex pathway composed of dozens of proteins that promote homologous recombination in fission yeast (*Schizosaccharomyces pombe*) by moving or pairing chromosomes, or forming or repairing DSBs. In a previous study, for example, Cromie *et al.* (2006) provided strong evidence that HJs in fission yeast are 'resolved' (*i.e.*, precisely cut and reconnected into two independent helices) solely by the endonucleolytic activity of the partner proteins Mus81 and Eme1. Although these proteins contain the active site for HJ resolution, the question still remained: How is HJ resolution by Mus81-Eme1 itself regulated?

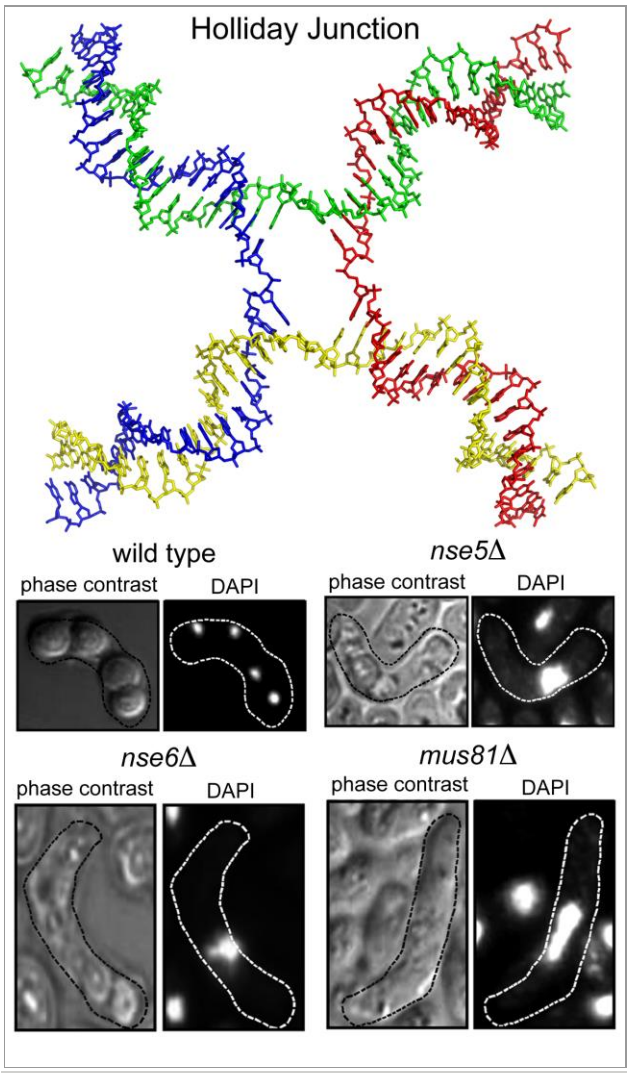
This question was answered in part through a recent collaborative study by first author Dr. Sophie Wehrkamp-Richter (The Scripps Research Institute), principal investigators Drs. Michael Boddy (Scripps) and Smith, and two additional co-authors, one of whom was Randy Hyppa of the Smith Lab. Previous work suggested that homologous recombination and HJ resolution in fission yeast might be influenced by a DNA repair complex composed of 'structural maintenance of chromosomes'

proteins 5 and 6 (Smc5-Smc6). Six essential non-Smc components of this repair complex have also been identified; these have been designated Nse1-Nse6. Wehrkamp-Richter *et al.*tested a variety of yeast mutants, including those in which the *nse5*, *nse6*, and/or *mus81* gene had been knocked out. They did so by means of genetic assays of DSB repair, biological assays of spore viability and morphology (*e.g.*, see figure), and physical assays for the presence of recombination intermediates known as joint molecules (JMs), at least some of which are HJs. The authors observed persistent JMs in mutant cells lacking Nse5 and Nse6. Elimination of Rec12 rescued the meiotic defects caused by deletion of *nse6* and *mus81*, demonstrating that Smc5-Smc6 affects fission yeast recombination *after* DSB formation. Similarly, heterologous expression of the bacterial HJ resolvase RusA partly rescued meiosis in *nse6*- or *mus81*-deficient yeast cells.

Taken together, these important findings demonstrate a novel regulatory role for the Smc5-Smc6 genome stability complex in HJ resolution via Mus81-Eme1. Future work will be needed to determine whether the Nse5 and Nse6 components of the Smc5-Smc6 holocomplex act directly in regulating HJ resolution or indirectly by recruiting the Smc5-Smc6 complex to its needed site of action.

Wehrkamp-Richter S, Hyppa RW, Prudden J, Smith GR, Boddy MN. 2012. Meiotic DNA joint molecule resolution depends on Nse5-Nse6 of the Smc5-Smc6 holocomplex. *Nucleic Acids Research*. Epub ahead of print, doi:10.1093/nar/gks713.

Also see: Cromie GA, Hyppa RW, Taylor AF, Zakharyevich K, Hunter N, Smith GR. 2006. Single Holliday junctions are intermediates of meiotic recombination. *Cell* 127:1167-1178.



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Schematic structure of a Holliday junction and mutations in fission yeast that result in aberrant asci (sexual, spore-bearing cells) due to problems with Holliday junction resolution. Above: The formation of one or more Holliday junctions, depicted here, is a necessary step in the recombination of genetic material during meiosis. Below: Spores in mature asci (outlined with dashed lines) are shown from crosses of wild type fission yeast, as well as crosses of strains in which the nse5, nse6 or mus81 gene has been knocked out. Due to problems with meiosis, each of these mutant lines forms unusual asci lacking well defined spores and/or containing only one large spore, rather than the normal complement of four smaller spores.