

An *elegans* Solution for Crossover Formation

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The MUS-81, SLX-1, and XPF-1 structure-selective endonucleases have been implicated in meiotic crossover (CO) formation in a variety of organisms, but their contributions to C. elegans CO formation have been unclear. In this issue of PLOS Genetics, Agostinho et al., Saito et al., and O'Neil et al. demonstrate that MUS-81 and XPF-1 function in two parallel pathways during the formation of meiotic crossovers in C. elegans and provide important insights into the interplay between endonucleases and the Bloom helicase ortholog during crossover formation.

Meiotic crossovers are important for chromosome segregation. When COs are absent or misplaced, nondisjunction results in aneuploidy, a leading cause of miscarriages and birth defects in humans. Our understanding of the complex process that generates COs has evolved steadily, and the models describing the molecular details have undergone much editing. Key features of the most commonly cited model for meiotic recombination include initiation by a DNA double-strand break (DSB) and a progression of joint molecule (JM) intermediates that link DNA duplexes on homologous chromosomes (reviewed in [1]). In this model, COs are produced by cleavage of a late JM intermediate that has two Holliday junctions (HJs). This cleavage is accomplished by HJ resolvases. A wealth of studies in the yeast *Saccharomyces cerevisiae* have provided extensive genetic and biochemical support for this model. Although initiation by DSBs appears to be universal (reviewed in [2]), it is unknown how similar subsequent steps are in other organisms. This issue of *PLOS Genetics* contains a set of three papers that describe progress toward answering this question in *C. elegans*, particularly with regard to functions of resolvases in generating COs [3–5] (Figure 1).

Numerous searches for *S. cerevisiae* enzymes with *in vitro* resolvase activity eventually identified three structure-selective endonucleases: Mus81–Mms4, Yen1, and Slx1–Slx4 [6–8]. Mus81–Mms4 and Yen1 are important in generating mitotic COs [9]. Elucidating the functions of these enzymes in generating meiotic crossovers has been more challenging, but substantial progress has been made recently [10,11]. Our current understanding is that there

are at least two CO pathways in *S. cerevisiae* meiosis (reviewed in [1]). The major pathway produces interfering COs, which are located farther apart than at random. The resolvase that generates these COs is thought to include Exo1 and the MutLγ complex, proteins that are also involved in mismatch repair [11]. Mus81–Mms4, Yen1, and Slx1–Slx4 do function in CO formation, but through a secondary pathway that yields non-interfering COs. Among single mutants, however, only *mus81* and *mms4* have reduced COs, and even loss of all three confers a relatively modest defect in JM resolution and CO formation, due to redundant and compensatory activities [10,11].

In the nematode *C. elegans*, previous work found that SLX-1 and the Slx4 ortholog HIM-18 are required for some meiotic COs [12]. These studies also found a role for the nucleotide excision repair endonuclease XPF-1, which was examined because of the prominent role for its *Drosophila* ortholog, MEI-9, in generating meiotic COs [13]. To shed additional light on the relationships between the proteins responsible for CO formation in *C. elegans*, Agostinho *et al.*, O'Neil *et al.*, and Saito *et al.* [3–5] quantified numerous meiotic phenotypes in single, double, triple, and even quadruple mutants. These studies led to the discovery of two parallel, partially redundant pathways—one dependent on MUS-81 and the other on XPF-1. Agostinho *et al.* and Saito *et al.* additionally demonstrate that SLX-1 functions with MUS-81 and that HIM-18 functions in both pathways, and Agostinho *et al.* provide evidence that HIM-6, the ortholog of the Bloom syndrome helicase, collaborates with XPF-1.

Saito *et al.* also extend a previous finding that SLX-1 actually prevents COs in the centers of the chromosomes [12].

What happens in the absence of both pathways? All three groups report multiple chromosome abnormalities. Most strikingly, connections between homologs persist into diakinesis, where they appear as fine DAPI-stained bridges. The researchers hypothesize that these bridges result from unresolved JMs. To test this hypothesis, Agostinho *et al.* and O'Neil *et al.* removed SPO-11, the enzyme that generates DSBs that initiate meiotic recombination. This eliminated chromatin bridges. In addition, O'Neil and colleagues elegantly show that the chromatin bridges in *mus-81;xpf-1* mutants are efficiently resolved by germline injection of human GEN1, which cleaves HJs *in vitro* [7], providing further support for the hypothesis that the bridges are due to unresolved HJs.

These studies further highlight the substantial complexity among resolvase functions in meiosis. Although *S. cerevisiae* resolvase functions are similarly complex, the details are different in many ways. First, in *S. cerevisiae*, Mus81 generates only non-interfering COs, but *C. elegans* MUS-81 seems to be involved in making interfering COs. Second, *S. cerevisiae* Rad1, the ortholog of XPF-1, has no apparent meiotic function [11]. Third, the JM-resolving activity of *S. cerevisiae* Slx1 and Slx4 is evident only in certain mutant backgrounds [10,11]. Fourth, in *S. cerevisiae*, there is no evidence that a resolvase can have an anti-CO role, as is suggested by Saito *et al.* for SLX-1 at the centers of chromosomes [4].

Additional potential similarities arise from studies of *him-6*, which encodes the ortholog of human Bloom syndrome

Citation: Bellendir SP, Sekelsky J (2013) An *elegans* Solution for Crossover Formation. *PLoS Genet* 9(7): e1003658. doi:10.1371/journal.pgen.1003658

Editor: Michael Lichten, National Cancer Institute, United States of America

Published: July 18, 2013

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Funding: This work was supported by the National Institute of General Medical Sciences of the National Institutes of Health (RO1 GM099890). The funder had no role in the preparation of the article.

Competing Interests: The authors have declared that no competing interests exist.

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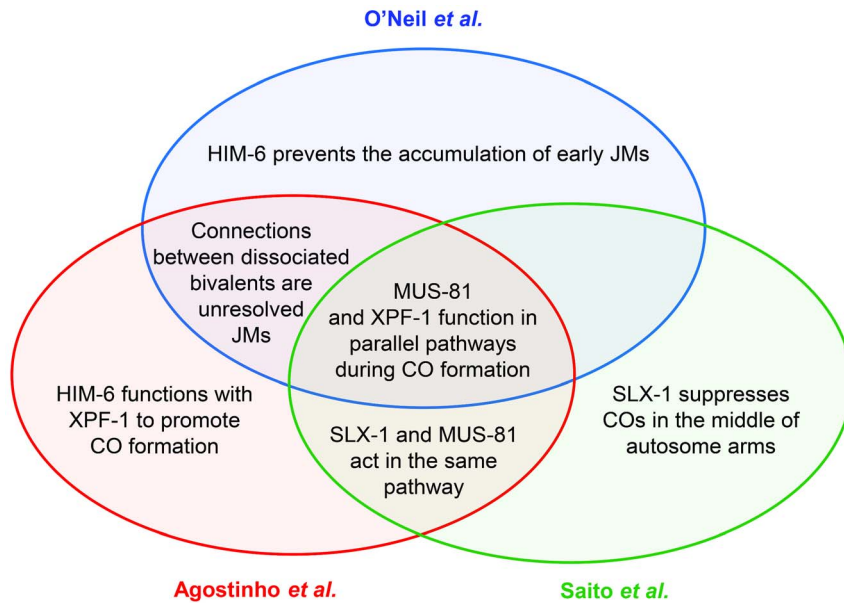


Figure 1. Venn diagram summarizing the main conclusions shared between the three publications discussed in the text.
doi:10.1371/journal.pgen.1003658.g001

helicase (BLM) and *S. cerevisiae* Sgs1. Sgs1 disassembles early meiotic JMs, both to generate noncrossovers and to prevent formation of aberrant multi-chromatid JMs [10,11]. O'Neil *et al.* report that *him-6* mutants have defects in processing early intermediates, leading to chromosome fragmentation [3]. Agostinho *et al.* propose that HIM-6 also has a late function in generating COs in conjunction with XPF-1 [5]. This is an exciting idea, especially

given the recent discovery of pro-CO roles for Sgs1 and *Drosophila* BLM [10,11,14], though the authors caution that a full understanding of this function is complicated by the earlier function for HIM-6.

Although the three groups began with similar goals, the different approaches taken in the three papers complement one another to provide numerous important insights into resolvase functions in *C. elegans* meiosis (Figure 1). The results raise

several important questions for future studies. First, what is the source of the COs that occur when both the MUS-81 and XPF-1 pathways are missing? Saito *et al.* and Agostinho *et al.* find that COs are reduced by only a little more than one third in *mus-81;xpf-1*. A simple interpretation is that neither of these enzymes identifies the major meiotic resolvase, but the truth is likely to be more complex. The assays used to measure COs necessarily select for a distinct subset of the progeny: those that had enough COs to ensure proper chromosome segregation. It is possible that the real decrease is more severe, and that MUS-81 and XPF-1 do define the major resolvases. Either way, there must be at least one additional resolvase that hasn't been identified. The authors of these papers discuss several candidates that might be tested.

Another important question concerns the exact nature of the pre-crossover JM. The authors suggest that these resolvases are acting on HJs, and resolution of chromatin bridges by GEN1 supports this suggestion. Agostinho *et al.* propose intriguing ideas for how the sets of enzymes (MUS-81, SLX-1, and HIM-18; and XPF-1, HIM-18, and HIM-6) might work together to resolve HJs. However, it is possible that the proteins act on a different structure, such as nicked HJs (*e.g.*, see reference [8]). Either way, the groundwork laid in this set of papers will facilitate future experiments aimed at answering these questions and many others.

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