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RESEARCH ARTICLE

# The mismatch repair and meiotic recombination endonuclease Mlh1-Mlh3 is activated by polymer formation and can cleave DNA substrates in trans

**Carol M. Manhart[1], Xiaodan Ni[2], Martin A. White[3], Joaquin Ortega[2], Jennifer A. Surtees[4], Eric Alani[1]***

**1** Department of Molecular Biology and Genetics, Cornell University, Ithaca, New York, United States of America, **2** Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario, Canada, **3** Department of Molecular and Cellular Biology, Harvard University, Cambridge, Massachusetts, United States of America, **4** Department of Biochemistry, University at Buffalo, State University of New York, Buffalo, New York, United States of America

* eea3@cornell.edu





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## Abstract

Crossing over between homologs is initiated in meiotic prophase by the formation of DNA double-strand breaks that occur throughout the genome. In the major interference-responsive crossover pathway in baker's yeast, these breaks are resected to form 3' single-strand tails that participate in a homology search, ultimately forming double Holliday junctions (dHJs) that primarily include both homologs. These dHJs are resolved by endonuclease activity to form exclusively crossovers, which are critical for proper homolog segregation in Meiosis I. Recent genetic, biochemical, and molecular studies in yeast are consistent with the hypothesis of Mlh1-Mlh3 DNA mismatch repair complex acting as the major endonuclease activity that resolves dHJs into crossovers. However, the mechanism by which the Mlh1-Mlh3 endonuclease is activated is unknown. Here, we provide evidence that Mlh1-Mlh3 does not behave like a structure-specific endonuclease but forms polymers required to generate nicks in DNA. This conclusion is supported by DNA binding studies performed with different-sized substrates that contain or lack polymerization barriers and endonuclease assays performed with varying ratios of endonuclease-deficient and endonuclease-proficient Mlh1-Mlh3. In addition, Mlh1-Mlh3 can generate religatable double-strand breaks and form an active nucleoprotein complex that can nick DNA substrates in trans. Together these observations argue that Mlh1-Mlh3 may not act like a canonical, RuvC-like Holliday junction resolvase and support a novel model in which Mlh1-Mlh3 is loaded onto DNA to form an activated polymer that cleaves DNA.

## Author summary

In sexually reproducing organisms, crossing over between homologous chromosomes in meiosis creates physical linkages required to segregate chromosomes into haploid