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Robust Crossover Assurance and Regulated Interhomolog Access Maintain Meiotic Crossover Number

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Abstract

Most organisms rely on interhomolog crossovers (COs) to ensure proper meiotic chromosome segregation but make few COs per chromosome pair. By monitoring repair events at a defined double-strand break (DSB) site during *Caenorhabditis elegans* meiosis, we reveal mechanisms that ensure formation of the obligate CO while limiting CO number. We find that CO is the preferred DSB repair outcome in the absence of inhibitory effects of other (nascent) recombination events. Thus, a single DSB per chromosome pair is largely sufficient to ensure CO formation. Further, we show that access to the homolog as a repair template is regulated, shutting down simultaneously for both CO and noncrossover (NCO) pathways. We propose that regulation of interhomolog access limits CO number and contributes to CO interference.

Crossover (CO) recombination during meiosis is initiated by induction of double strand breaks (DSBs) (1). Interhomolog (IH) COs provide connections (chiasmata) that enable homologous chromosomes to orient and segregate to opposite poles at the meiosis I division (2). To ensure formation of IH COs, DSB repair is modulated to (i) promote use of the homologous chromosome as a repair template, as opposed to the sister chromatid (the preferred template in other contexts), and (ii) promote maturation of IH recombination intermediates via a pathway that yields CO rather than noncrossover (NCO) products (3, 4). However, more DSBs are formed than IH COs, indicating that excess DSBs must be repaired in other ways (3, 4). Moreover, COs inhibit the formation of other COs nearby on the same chromosome, a phenomenon known as CO interference (5). Thus, successful meiosis requires a balance between mechanisms that promote and inhibit formation of IH COs.

To investigate regulation of meiotic COs, we monitored outcomes of IH DSB repair events at a defined locus in the *Caenorhabditis elegans* genome [Fig. 1A and supporting online material (SOM) text] (6, 7). DSBs are generated by heat shock–induced excision of a *Mos1*

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Supporting Online Material

www.sciencemag.org/cgi/content/full/334/6060/1286/DC1

Materials and Methods

SOM Text

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transposon from a unique insertion site that disrupts the *unc-5* gene (6). Repair using the homolog, which contains a different *unc-5* mutation, results in restoration of the wild-type (WT) *unc-5(+)* gene. Both CO and NCO repair outcomes are assessed, and repair type can be distinguished by using a linked marker (7) (Fig. 1A). We exploited the production-line organization of the *C. elegans* germ line (fig. S1) to assess competence for IH repair and for CO versus NCO outcome for germ cells at different stages of meiotic prophase progression at the time of heat shock. We conducted time-course experiments in which young adult hermaphrodites were heat-shocked and subsequently transferred to fresh plates at specified time points. Cohorts of progeny arising from eggs laid during each interval were scored for repair events (Fig. 1B). Progeny from eggs laid 10 to 22 hours post heat shock (phs) did not contain any *unc-5(+)* recombinants, indicating that no IH repair had occurred in the corresponding parental germline nuclei, which were furthest in meiotic prophase progression at the time of heat shock. Recombinants reflecting IH repair events first appeared in the next interval (22 to 34 hours phs), at a frequency of 2%, followed by a consistent frequency of 7% in subsequent cohorts. As soon as IH recombinants appeared, both CO and NCO products were seen. Further, the CO:NCO ratio remained constant throughout the time course, with COs representing ~11% of IH recombinants (Fig. 1B). We determined that progeny from the 22- to 34-hour cohort were derived from nuclei that had completed assembly of the chromosome-associated synaptonemal complex (SC) before the time of heat shock (8) (SOM text and fig. S2). Thus, we infer that DSBs induced after SC assembly can be competent to become COs. Further, the constant CO:NCO ratio implies that access to the homolog as a repair template is shut down simultaneously for both CO and NCO pathways.

During *C. elegans* meiosis, robust interference results in each chromosome pair having a single CO (9). By using a modified version of the assay, we tested whether *Mos1*-induced COs confer interference. Introgression of *unc-5(e791)* into a different strain background allowed assessment of COs elsewhere along the chromosome by scoring of single nucleotide polymorphism (SNP) markers (Fig. 2A) (8). On chromosomes with an NCO event at the *Mos1* site, we readily detected COs elsewhere on the chromosome at a frequency (38%) commensurate with expectations based on the genetic map (Fig. 2A). In contrast, on chromosomes with a CO event at the *Mos1* site, no additional COs were detected ($P < 0.0001$). Thus, like endogenous COs, *Mos1*-induced COs inhibit other COs on the chromosome. These data, coupled with the constant CO:NCO ratio, imply that DSBs induced after SC assembly can compete with endogenous DSBs to become the sole CO. This conclusion is incompatible with a model in which CO/NCO decisions are irrevocably made before SC assembly, as is proposed to occur in budding yeast (10). Our data support a model in which several IH interactions are established, and among these interactions a CO site is subsequently specified in the context of assembled SC.

We also assessed DSB repair outcomes when the *Mos1* site is the sole DSB, by using the *spo-11* mutant, which is proficient for pairing and SC assembly but lacks endogenous DSBs and COs (11). The overall frequency of *unc-5(+)* recombinants in the *spo-11* background was similar to that observed in WT. However, whereas only ~11% of events were COs in WT, almost all IH repair events in the *spo-11* mutant were COs (Fig. 3A; $P < 0.0001$). This indicates that CO is the preferred outcome in the absence of inhibitory effects from events initiated at other DSBs. Efficient induction of COs in the *spo-11* mutant was also demonstrated by cytological detection of chiasmata in diakinesis-stage oocyte nuclei of heat-shocked worms (Fig. 3, B and C). The estimated incidence of chiasmata (9 to 20%) corresponds well with the frequency of recombinants observed genetically (Fig. 3C and SOM text). Our results show that a single DSB is converted to a CO with high efficiency. Thus, although most chromosome pairs undergo multiple meiotic DSBs (SOM text and fig. S4), CO assurance can be achieved largely by ensuring that each chromosome pair receives at least one DSB.

Msh5 is a meiosis-specific MutS homolog that has a conserved role in promoting COs (4, 12); in *C. elegans*, essentially all COs are MSH-5–dependent (13). We asked whether MSH-5 is required specifically for the CO fate or whether it might be required for all IH recombination. We detected *unc-5(+)* recombinants in the *msh-5* background at frequencies comparable to WT (Fig. 4A), indicating that the mutant is proficient for IH repair. As expected (13), all observed events were NCOs (Fig. 4B). Thus, MSH-5 is not required to establish IH recombination interactions but instead acts specifically to promote the CO outcome.

RTEL-1 is a helicase previously shown to limit CO number during *C. elegans* meiosis (14, 15). RTEL-1 was proposed to act in executing CO/NCO decisions by promoting the NCO outcome. It was further suggested that, in the absence of RTEL-1, all DSBs are repaired as COs (15). Contrary to this prediction, we found that NCO repair occurs in the *rtel-1* mutant background. In 13 of 14 cases analyzed, *unc-5(+)* recombinants recovered in the *rtel-1* mutant background were NCOs (Fig. 4B). Thus, RTEL-1 is not strictly required for the NCO repair outcome and may limit COs in other ways.

In both the *msh-5* and *rtel-1* time courses, we detected *unc-5(+)* IH recombinants among progeny from the earliest time interval (10 to 22 hours phs), whereas recombinants were never recovered from this interval in WT time courses (Fig. 4A; $P = 0.007$ for *msh-5* versus WT, $P = 0.003$ for *rtel-1* versus WT). This implies that, both in the absence of MSH-5 (which is required to form COs) and in the absence of RTEL-1 (which is required to limit COs), access to the homolog as a repair template is significantly prolonged.

Our analysis addresses two fundamental aspects of CO regulation: the ability to ensure a CO for each homolog pair (CO assurance) while at the same time limiting their number (CO interference). A phenomenon known as “crossover homeostasis” has been described in *Saccharomyces cerevisiae*, where under conditions of reduced DSBs, the number of COs was maintained while NCOs decreased (16, 17). We demonstrate that CO homeostasis is extremely robust in *C. elegans*. When only one break is formed, CO is the heavily favored repair outcome. This implies that CO assurance can be achieved largely by ensuring that each chromosome pair receives a DSB. We also uncovered a separate level of regulation, namely the restriction of access to the homologous chromosome as a repair template. Although IH interactions are essential to form COs, we propose that access to the homolog is dynamically regulated during meiosis, with IH access turned on to ensure the obligate crossover, then turned off to limit CO number, thereby contributing to the implementation of CO interference (fig. S5). Our data suggest that germ cells can monitor whether sufficient “CO-eligible” IH interactions have been established to guarantee a CO. Once this condition is met, further breaks are inhibited from engaging in IH repair. The data further suggest that CO designation occurs either concurrently with or subsequent to IH shutdown, ultimately resulting in a subset of the established IH interactions being processed into COs. In the context of this model, prolonged IH access in *msh-5* mutants might reflect a role for MSH-5 in generation and/or surveillance of CO-eligible IH-recombination intermediates. Further, prolonged IH access in the *rtel-1* mutant suggests a revised view regarding the role of RTEL-1 in inhibiting excess COs: We propose that in addition to (or instead of) disengaging existing IH recombination intermediates, RTEL-1 acts as an effector in shutting down IH access by antagonizing formation of new IH strand invasion intermediates.

Our analysis highlights the potential for mechanisms that regulate the duration of IH access to play a substantial role in shaping the CO landscape. Interestingly, a recent study showed evidence that different genomic regions exhibit different repair partner preferences (IH versus intersister) during *Schizosaccharomyces pombe* meiosis (18), suggesting that IH

access can also be restricted spatially. Thus, mechanisms that modulate IH access may be important determinants of CO frequency and distribution in many organisms.

Our work also provides insights regarding how transposons interact with host genomes. DSBs induced by *Mos1* excision are processed into COs with properties similar to endogenous COs, indicating that transposons can contribute to genome evolution by inducing COs. However, transposon-induced DSBs compete with endogenous DSBs for CO designation and are subject to CO interference, indicating that endogenous recombination events can mitigate the effect of transposons in reshaping the genome. This raises the possibility that crossover interference may have evolved in part to limit the impact of transposons on genome evolution.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Fig. 1. Meiotic recombination initiated by *Mos1* excision-induced DSBs. **(A)** *Mos1* excision-induced DSB repair assay. **(B)** Time-course analysis of IH recombination induced at the *Mos1* site. Chart shows the incidence of *unc-5(+)* recombinants among progeny derived from eggs laid in the indicated time intervals (hours phs) and the incidence of CO and NCO repair types (8) (table S3).

Fig. 2. COs induced by *Mos1* excision inhibit formation of other COs on the same chromosome. Modified *Mos1* DSB repair assay to evaluate CO interference. Purple indicates N2-derived chromosome IV segments, orange indicates segments derived from the Hawaiian strain background, and ticks indicate genetic positions of SNP markers. Genotypic classes (and number of occurrences) are shown for chromosomes that had undergone either a CO or NCO event at *Mos1*.

Fig. 3.

Mos1-induced DSBs are efficiently converted into COs when endogenous DSBs are absent. (A) IH recombinants and CO versus NCO repair types after DSB induction at the *Mos1* site in *spo-11* worms. Here and in Fig. 4, a subset of recombinants could not be scored for CO versus NCO repair type because of premature death or aneuploidy (8). (B) *Mos1*-initiated COs in the *spo-11* background result in cytologically detectable chiasmata. (Top) The full complement of chromosomes in a single diakinesis-stage oocyte nucleus. (Left) WT nucleus with six pairs of homologs connected by chiasmata; four are oriented so that chromosome axis protein HIM-3 is visible in a cross-shaped structure marking the chiasma. (Center and right) Nuclei from a *spo-11* mutant carrying *unc-5(Mos1)*, 40 hours after heat-shock induction of transposase; the center nucleus has 12 unattached chromosomes (univalents), whereas the left nucleus contains one bivalent with a *Mos1*-induced chiasma (arrowhead). (Bottom) Single focal plane highlighting the chiasma and a nearby univalent. (C) Quantitation of chiasma formation elicited by heat shock-induced DSBs; parentheses indicate numbers of nuclei scored. Detection of nuclei with unambiguous chiasmata after DSB induction is highly significant, because these were never detected in controls (* $P=0.001$). The 11 DAPI bodies (ambiguous) class can include both (i) nuclei with two univalents in close proximity that are difficult to resolve and (ii) nuclei with a real CO/chiasma in which the chiasma is in an unclear orientation. DAPI, 4',6-diamidino-2-phenylindole.

A

Egg-laying period (Hours phs)	Unc-5(+) Recombinant / Total Progeny		
	WT	<i>msh-5</i>	<i>rtel-1</i>
10-22	0/ 1414 (0%)	2/ 107 (2%) *	4/ 332 (1%) **
22-46	153/ 3459 (4%)	21/ 331 (6%)	9/ 436 (2%)
46-70	103/ 1403 (7%)	11/ 205 (5%)	4/ 75 (5%)
70+	10/ 169 (6%)	6/ 55 (11%)	n/a
Total:	266/ 6445 (4%)	40/ 698 (6%)	17/ 843 (2%)

B

	WT	<i>msh-5</i>	<i>rtel-1</i>
CO : NCO	28 : 228	0 : 25	1 : 13

Fig. 4.

Time-course analysis of *MosI*-induced meiotic IH recombination in *msh-5* and *rtel-1* mutants. **(A)** Incidence of *unc-5(+)* recombinants among progeny derived from eggs laid in the indicated time intervals phs; *unc-5(+)* recombinants were never detected in the 10- to 22-hour interval (corresponding to nuclei furthest in meiotic progression at the time of heat shock) in WT but were detected in this early interval in both *msh-5* (* $P=0.007$) and *rtel-1* (** $P=0.003$) mutant backgrounds. **(B)** Incidence of CO and NCO repair types (8).