Crossing Over During *Caenorhabditis elegans* Meiosis Requires a Conserved MutS-Based Pathway That Is Partially Dispensable in Budding Yeast

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ABSTRACT

Formation of crossovers between homologous chromosomes during *Caenorhabditis elegans* meiosis requires the *him-14* gene. Loss of *him-14* function severely reduces crossing over, resulting in lack of chiasmata between homologs and consequent missegregation. Cytological analysis showing that homologs are paired and aligned in *him-14* pachytene nuclei, together with temperature-shift experiments showing that *him-14* functions during the pachytene stage, indicate that *him-14* is not needed to establish pairing or synapsis and likely has a more direct role in crossover formation. *him-14* encodes a germline-specific member of the MutS family of DNA mismatch repair (MMR) proteins. *him-14* has no apparent role in MMR, but like its *Saccharomyces cerevisiae* ortholog *MSH4*, has a specialized role in promoting crossing over during meiosis. Despite this conservation, worms and yeast differ significantly in their reliance on this pathway: whereas worms use this pathway to generate most, if not all, crossovers, yeast still form 30–50% of their normal number of crossovers when this pathway is absent. This differential reliance may reflect differential stability of crossover-competent recombination intermediates, or alternatively, the presence of two different pathways for crossover formation in yeast, only one of which predominates during nematode meiosis. We discuss a model in which HIM-14 promotes crossing over by interfering with Holliday junction branch migration.

FAITHFUL segregation of homologous chromosomes at the meiosis I division is dependent on the formation of crossovers between the two homologs (Hawley 1988). Crossing over leads to the formation of chiasmata, temporary physical connections that hold homologs together until anaphase of meiosis I and allow the homologs to orient toward opposite spindle poles (Nicklas 1974; Jones 1987). These connections are particularly crucial in organisms that undergo a classical prolonged meiotic prophase and pause or arrest prior to assembling a meiosis I spindle. In such organisms the connections between homologs afforded by the chiasmata must persist for many hours, days, or even years; if chiasmata fail to form or are prematurely released, the homologs lose spatial relationship to each other, making it impossible for them to become oriented on the spindle in a way that will ensure their segregation to opposite poles.

Given the importance of meiotic crossovers for segregating chromosomes, however, most organisms actually make very few of them. Genetically well-studied eukaryotes vary widely in haploid chromosome number, over an order of magnitude in the sizes of their genetic maps, over two orders of magnitude in genome size, and over three orders of magnitude in amount of DNA/genetic map unit. Morever, there is absolutely no correlation between chromosome number and genome size, nor between the frequency of recombination/unit DNA and genetic map size. Virtually the only major genomic parameter that is widely conserved is the number of crossovers per chromosome pair, usually no more than one to three crossovers/chromosome arm. Thus crossing over must be carefully regulated to ensure that a limited number of events is distributed in a way that guarantees each chromosome pair will have a crossover.

The nematode *C. elegans* represents an extreme example of the general case, usually undergoing only one crossover per chromosome pair per meiosis (Brenner 1974; Barnes *et al.* 1995). This is not simply an average of one crossover, since cytological analysis of oocyte meiosis shows that achiasmate chromosomes are extremely rare (Villeneuve 1994; Dernburg *et al.* 1998), in contrast to the high frequency of achiasmate chromosomes predicted if crossover events followed a Poisson distribution. Thus meiotic chromosome segregation in nematodes is particularly sensitive to alterations in either the number or distribution of crossovers (reviewed

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in Albertson *et al.* 1997), making this system well suited for genetic analysis of both the mechanism of crossover formation and its regulation. Further, the organization of meiotic nuclei in the nematode germline permits rapid cytological assessment of defects in crossover formation even in mutants that produce very few or no viable progeny (Schedl 1997; Dernburg *et al.* 1998). Finally, prior initiation of recombination is not required in the nematode for formation of the meiosis-specific synaptonemal complex between paired aligned homologous chromosomes, making it possible to examine the consequences of defects in recombination machinery in the absence of concomitant defects in synapsis (Dernburg *et al.* 1998).

We report here our analysis of *C. elegans him-14*, which encodes a germline-specific member of the MutS protein family that is required during the pachytene stage of meiotic prophase for the formation of meiotic crossovers. A recurring two-part theme emerges from this and other recent work on meiotic recombination in the nematode: (1) conserved pathways for meiotic recombination are used in highly diverged organisms, but (2) despite this conservation, diverged organisms differ substantially in the ways that they utilize and rely on conserved recombination components. Thus the study of meiotic recombination in *C. elegans* contributes both to understanding and defining the essential features of meiosis, and to revealing the ways in which these features may be modified to function in the context of highly diverged cellular architectures and physiologies.

MATERIALS AND METHODS

Strains and maintenance: General methods for culturing *C. elegans* strains were as described in Brenner (1974), Wood (1988), and Epstein and Shakes (1995). Except where noted, all experiments were performed at 20°. The following mutations, polymorphisms, and chromosome rearrangements were used (Riddle *et al.* 1997):

LGII: unc-4(e120), unc-104(e1265), him-14(it13, it21, it23, it44ts, me15), pkP504, lin-26(n156), rol-6(e187), mnDf30, mnDf105, mnDf88, mnC1
LGIV: unc-5(e53), dpy-20(e1282ts)
LGX: unc-1(e1598n1201), dpy-3(e27), xol-1(y9), unc-3(e151).

Some strains were kindly provided by the Caenorhabditis Genetics Center.

Detection of achiasmate chromosomes in oocyte nuclei: To assess frequencies of achiasmate oocyte chromosomes, worms were fixed with Carnoy's fixative and stained with 4′,6-diamidino-2-phenylindole (DAPI) as described in Villeneuve (1994). In some nuclei, individual univalents or bivalents may lie too close to each other to be resolved unambiguously; thus this method underestimates the frequency of achiasmate chromosomes.

Imaging of meiotic chromosome morphology: Preparation of worms for cytological analysis was carried out with modifications of procedures described in Dernburg *et al.* (1998). Gravid adult hermaphrodites were dissected in 10 μl of 1× egg buffer [118 mm NaCl, 48 mm KCl, 2 mm $CaCl_2$, 2 mm $MgCl_2$, 5 mm HEPES (pH 7.4) (Edgar 1995)], on a glass coverslip. Worms were sliced between two 25-gauge needles near the head and the tail to release each arm of the gonad, then washed into 1.5 ml Eppendorf tubes with 1 ml 3.7% formaldehyde in 1× egg buffer (diluted from fresh 37% formaldehyde). Fixation was carried out for 20 min, followed by five washes in 2× SSCT (0.3 m NaCl, 0.03 m Na citrate, 0.1% Tween-20). Worms were held in 2 μg/ml DAPI (Sigma, St. Louis) for 5 min, then washed in 2× SSCT seven times. Worms were mounted in 90% (glycerol + 4% *N*-propyl gallate) 0.12 m Tris buffer. Imaging of DAPI-stained chromosomes was carried out using the Deltavision deconvolution microscopy system (Applied Precision) as described in Dernburg *et al.* (1998).

Fixation for *in situ* hybridization: Animals were dissected as above, but then an equal volume of 7.4% formaldehyde in 1× egg buffer (diluted from fresh 37% formaldehyde) was added to the worms on the coverslip. A SuperFrost Plus slide (Fisher) was touched to the worm/fix drop to pick up the coverslip, and the slide was immediately placed flat on the bottom of an ice bucket containing liquid N_2. A razor blade was used to crack the coverslip off of the slide, which was then transferred to 95% ethanol at −20°. The ethanol slides were brought to room temperature, and the slides were gradually rehydrated through the following series of washes: 3:1 (95% ethanol: 2× SSCT), 1:1 (95% ethanol: 2× SSCT), 1:3 (95% ethanol: 2× SSCT), then 3× in 100% SSCT. Fluorescence *in situ* hybridization (FISH) was performed as in Dernburg and Sedat (1998).

Probes for *in situ* hybridization: The 5S rDNA probe was a gift of Abby Dernburg (Dernburg *et al.* 1998). The Y22E4 probe was generated by degenerate oligonucleotide-primed PCR (DOP-PCR; Telenius *et al.* 1992) as described by Albertson *et al.* (1995), with modifications. The degenerate oligo primer set used was the following: CCGACTGAGNNNNN NATGTGG (Telenius *et al.* 1992). DNA from YAC clone Y22E4 (kindly provided by Dr. Alan Coulson and the *C. elegans* Sequencing Consortium at the Sanger Centre) was separated from yeast chromosomes by CHEF gel electrophoresis (Vollrath and Davis 1987). A gel slice containing the YAC was melted at 65° and used immediately as template for the PCR reaction, as follows: 4 min at 93°; 3 cycles of (30 sec at 94°, 1 min at 30°, ramp to 72°, over 5 min, hold 1 sec); 3 cycles of (30 min at 94°, 1 min at 30°, 2 min at 72°); 36 cycles of (20 sec at 94°, 1 min at 56°, 2 min at 72°); 10 min at 72°. The PCR products were digested using a mixture of 4-base-cutter restriction enzymes, then 3′ end labeled with Cy3-dCTP (Amersham, Piscataway, NJ) as described by Dernburg and Sedat (1998).

Genetic recombination frequencies: Males of genotype *him-14(it21)/mnC1* were crossed with hermaphrodites of genotype: *him-14(it21)/mnC1; dpy-3 unc-3* or *him-14(it21)/mnC1; unc-1 dpy-3* or *him-14(it21)/mnC1; unc-5 dpy-20*. (The *unc-5 dpy-20* experiment was performed at 23°.) Cross-progeny hermaphrodites were picked to single plates and transferred daily for 2 days, and complete broods were scored for UncDpy, wild-type, and Unc non-Dpy, and Dpy non-Unc recombinant progeny. *him-14/him-14* animals were distinguished from *him-14/mnC1* animals based on production of inviable zygotes *vs.* production of *mnC1* homozygotes; *mnC1* homozygotes were excluded from scoring. Recombination frequencies (p) were calculated as $p = 1 - (1 - 2R)^{1/2}$, where R is the frequency of phenotypic recombinant progeny (Brenner 1974). Since $p = R$ for small p, numbers for both male and hermaphrodite progeny were combined for *him-14* homozygote data for *X* chromosome and autosomal intervals. Map distances in centimorgans = $100 \times p$.

Temperature-shift experiments: For the upshift, newly gravid young adult hermaphrodites grown at 15° were picked 20/plate, and plates were shifted to 23°. For the downshift, newly gravid young adult hermaphrodites grown at 23° were

picked 20/plate, and plates were shifted to 15°. At each time point, worms from a single plate were transferred to a slide and fixed with Carnoy's fixative (Villeneuve 1994). Worms were stained with DAPI and the number of DAPI-staining bodies in oocyte nuclei was assessed (50–150 nuclei were scored for each time point). In most nuclei that had achiasmate chromosomes, 11–12 DAPI-staining bodies were resolved.

Gravid adult hermaphrodites have ~300–350 pachytene and 10–12 postpachytene nuclei per gonad arm (Austin and Kimble 1987; White 1988; Schedl 1997; Dernburg *et al.* 1998). A rough estimate has been made that half of these pachytene nuclei will eventually undergo apoptosis at the end of the pachytene stage (Gumienny *et al.* 1999). We have used the very conservative assumption that as many as 80% of pachytene nuclei will undergo apoptosis to estimate that 70–80 nuclei (that will survive to be oocytes) per gonad arm will be at pachytene or later at the time of the shift. Progression of nuclei through meiosis is coupled to the rate of egg-laying. Under the conditions used, 2–2.5 eggs/hr/gonad arm are laid following the upshift, and 1–1.3 eggs/hr/gonad arm are laid following the downshift. Thus we can infer that nuclei from the 17.7- and 21.6-hr upshift time points (which exhibit a high frequency of achiasmate chromosomes) were in the pachytene stage at the time of the shift. Further, we can infer that nuclei from the 36-hr downshift time point (many of which exhibit full rescue) had already reached the pachytene stage at the time of shift. It is probable that 59-hr time point nuclei were also at pachytene, but this cannot be established unambiguously owing to uncertainty inherent in estimating apoptosis rates (Gumienny *et al.* 1999).

Genetic mapping: The high resolution map position of *him-14* was determined by a combination of multi-point crosses and deficiency mapping, using the *it44* allele. Multi-point mapping results are summarized below; for each heterozygote shown, the number of the total recombinant progeny selected that had a crossover in a given interval is shown in parentheses between the markers flanking that interval:

unc-104 rol-6/him-14: *unc-104* (1/65) *him-14* (64/65) *rol-6*
him-14 rol-6/pkP504: *him-14* (3/15) *pk504* (12/15) *rol-6*
unc-104 lin-26/him-14: *unc-104* (2/18) *him-14* (16/18) *lin-26*.

The breakpoints of *mnDf10* and *mnDf88* were mapped previously to cosmid T05A9 (Labouesse *et al.* 1994). We mapped the breakpoint of *mnDf30* to cosmid ZK1127 by using PCR to test for the presence or absence of DNA from the region in inviable embryos homozygous for *mnDf30*. Single embryo PCR reactions were carried out as described by Williams *et al.* (1992), with buffer and temperature conditions optimized for specific sets of primers. Multiplex PCR reactions contained both positive (target present in *mnDf30*) and negative (target should be absent in *mnDf30*) control primers. A primer pair corresponding to coordinates 6744-6908 in the ZK1127 sequence failed to amplify a product from *mnDf30* homozygotes, whereas primer pairs corresponding to coordinates 30841-31148 and coordinates 35045-35330 did amplify products from *mnDf30* homozygotes, indicating that the breakpoint of *mnDf30* is between coordinates 6744 and 30841 of cosmid ZK1127.11.

Northern analysis and *him-14* cDNA: A 2-kb PCR fragment derived from a composite cDNA from the predicted gene ZK1127.11 (see below) was labeled with [^{32}P]dATP by random priming (Sambrook *et al.* 1989) and used to probe a Northern blot containing RNA from wild-type adults and *glp-4(bn2)* adults lacking a germline as described in Dernburg *et al.* (1998). The same blot was probed separately using a genomic PCR fragment to detect a control transcript from the adjacent gene T02G5.9, which encodes a lysyl tRNA synthetase.

A 1.6-kb partial cDNA clone from the predicted gene ZK1127.11, yk240h12, was obtained from Dr. Yuji Kohara of the National Institute of Genetics, Mishima, Japan. cDNA corresponding to the 5′ end of the *him-14* transcript was obtained by RT-PCR, in two steps. In the first step, first-strand cDNA synthesis was primed using the gene-specific primer GTGATG GCGATTCCTTCTTC, and the cDNA was amplified using a nested primer, TCAGCGATCTCTCGTGCTCC, in conjunction with the primer CGGCCTCGAGATGAATGCAGAAGTT TCGACGG, corresponding to the initiation codon predicted by Genefinder plus an added *Xho*I site. In the second step, first-strand cDNA synthesis was primed using the gene-specific primer TCCAGCCATATTTGGACCCG, and cDNA corresponding to the 5′ end of the transcript was amplified using this primer in conjunction with the primer GGTTTAATTACC CAAGTTTGAG corresponding to the *trans*-spliced leader SL1. The assembled composite cDNA is 2.6 kb in length (excluding poly(A) tail), corresponding to the size of the single transcript detected by Northern analysis.

Identification of molecular lesions in *him-14* alleles: Single-stranded conformation polymorphism (SSCP) analysis was used to identify DNA fragments containing mutations in *him-14* mutant alleles. A series of 300–500 bp [^{32}P]dATP-labeled PCR products encompassing the entire coding sequence and all intron/exon boundaries was generated using lysed single wild-type or homozygous mutant worms as a source of genomic DNA template. Because a 1.1-kb segment of this gene is duplicated elsewhere in the genome, a 1.3-kb unlabeled PCR fragment from this region was generated first, using one primer from outside the duplicated region and one from within it; this 1.3-kb fragment was then used as template to generate labeled smaller fragments for SSCP analysis. Fragments were separated and analyzed using the MDE gel system from FMC (Rockland, ME, catalog 50620) according to the manufacturer's protocol, and fragments of altered mobility were identified. Sequencing to pinpoint the lesions identified by the SSCP analysis was carried out using the USB Sequenase 2.0 DNA sequencing kit (70770).

Measurement of spontaneous mutation frequency: We measured the frequency of spontaneous mutations conferring levamisole resistance (LevR) in the germlines of *him-14(it44); xol-1(y9)* worms and *xol-1(y9)* control worms. Most LevR mutations are recessive, so the frequency of spontaneous mutations present in the germline was assayed by scoring the phenotype in the F_2 generation. The *xol-1(y9)* mutation was included to eliminate males and thereby ensure inbreeding, allowing newly arising LevR mutations to become homozygous.

For both genotypes, each of six independent 250-ml liquid cultures was inoculated with freshly starved worms washed off in a 100-mm nematode growth medium plate grown to starvation at 15°. Cultures were grown at 15°, and 2 days after hermaphrodites that had been L1 larvae at inoculation became gravid, embryos were harvested by hypochlorite treatment and allowed to hatch in the absence of food to synchronize them as L1 larvae (which have only two germline precursor cells) (Epstein and Shakes 1995). Approximately 3×10^6 synchronized L1 larvae were harvested from each culture and used to inoculate fresh 250-ml liquid cultures, which were grown at 23° until the worms had been gravid adults for 1 day. These F_1 embryos were harvested and synchronized at the L1 stage as before. F_1 worms were then plated at 15° at a density of 10,000 worms/plate and allowed to produce F_2 progeny. Since the plates contain roughly 120,000 worms at the point of starvation, we estimate that when the plates starve, 95% of the chromosomes present in the F_1 animals are represented in homozygotes at least once; thus ~19,000 gamete equivalents are tested per plate. F_2 worms were washed off plates and transferred to plates containing 1 mm levamisole

at 15°; plates were screened several days later for the presence of fast-growing or fast-moving worms that were not hypercontracted (Lewis *et al.* 1980).

RESULTS

***him-14* is required for crossover formation during *C. elegans* meiosis:** To identify components of the cellular machinery required for the formation and regulation of meiotic crossovers, we screened for *C. elegans* mutants defective in meiotic chromosome segregation (Villeneuve 1994). The screening strategy exploits the chromosomal sex-determination system of this nematode. *C. elegans* exists as two sexes: a hermaphrodite, which has two sex chromosomes (*XX*), and a male, which has one sex chromosome (*X*O). Hermaphrodites make sperm briefly before switching to oogenesis, and thus can reproduce either by self-fertilization or by cross-fertilization. A hermaphrodite reproducing by self-fertilization produces broods consisting almost entirely of hermaphrodites, but males arise among hermaphrodite self-progeny at a frequency of 0.2% owing to spontaneous errors in chromosome segregation during meiosis (Hodgkin *et al.* 1979). Thus, mutants exhibiting defects in meiotic chromosome segregation were sought by screening for healthy, anatomically normal hermaphrodites that produced an increased frequency of self-progeny males. A number of mutants of this type have been described previously, and have been termed "Him" mutants, for *h*igh *i*ncidence of *m*ales (Hodgkin *et al.* 1979).

A major class of mutants arising in our screen are defective for the segregation of all chromosomes. These mutants produce broods consisting mainly of inviable aneuploid embryos that arrest with variable morphology, but they also produce a few healthy, fertile, anatomically normal adult survivors, many of which are male, that by chance received a euploid (or near euploid) chromosome complement. Mapping and complementation tests showed that one mutation conferring this phenotype, *me15*, is an allele of the previously identified gene *him-14*. Five other *him-14* alleles were isolated in a screen for maternal-effect lethal mutations on chromosome *II* (Kemphues *et al.* 1988); these mutations masquerade as apparent maternal-effect lethals since their meiotic defect results in broods comprised mainly of dead embryos. Hermaphrodites homozygous for the presumptive null allele *it21* (see below) typify the phenotype of *him-14* mutants: they produce nearly normal numbers of embryos, but 97% of these fail to hatch (n = 3777); among the progeny that do survive to adulthood, 45% are male, indicative of a severe chromosome segregation defect.

Cytological examination of DAPI-stained chromosomes late in meiotic prophase reveals an absence of chiasmata in *him-14* mutant oocytes that readily accounts for the observed chromosome segregation defect. *C. elegans* oocyte nuclei pause or arrest late in meiotic prophase, at the diakinesis stage (Schedl 1997). By this time the homologs have lost the side-by-side association seen at earlier stages, but homologs remain attached to each other by chiasmata, the cytological manifestation of earlier crossover events. In wild-type oocytes, 6 DAPI-stained bodies are observed, corresponding to the six pairs of homologs attached by chiasmata (Figure 1a; Villeneuve 1994; Dernburg *et al.* 1998). In *him-14* mutants, in contrast, 12 discrete DAPI-stained bodies could be clearly resolved in the majority of oocyte nuclei, indicating an absence of chiasmata (Figure 1b). Specifically, an average of 11.6 DAPI-staining bodies were resolved in 147 nuclei from 21 hermaphrodites, indicating that most, if not all, chromosomes lack chiasmata.

Measurement of genetic recombination frequencies in *him-14* mutants indicates that the absence of chiasmata at diakinesis is due to a failure to form crossovers (and therefore chiasmata) rather than to premature release of chiasmata that had already formed. For an interval encompassing 80% of the *X* chromosome, the crossover frequency in *him-14(it21)* homozygous hermaphrodites was reduced to <1% of the wild-type level (Table 1). Crossovers were also not detected in the *him-14(it21)* mutant for the two other intervals tested, including one on an autosome (Table 1). These data demonstrate that *him-14* is required for the formation of most, if not all, meiotic crossovers. This finding extends a previous observation of a 50% reduction in recombination frequencies in the temperature-sensitive mutant *him-14(it44ts)* at semipermissive temperature (Zetka and Rose 1995). Failure to detect crossovers in these experiments indicates that spermatocyte meiosis as well as oocyte meiosis is defective in *him-14* mutant hermaphrodites, corroborating our cytological observations of rampant missegregation of chromosomes during spermatocyte meiosis in both males and hermaphrodites (data not shown).

Intimate pairing and homolog alignment are normal in *him-14* mutants: *A priori*, failure to form crossovers could be caused by defects in the recombination machinery itself, in the regulation of recombination events, or in events that are prerequisites for successful regulated recombination, such as the pairing and alignment of homologous chromosomes. The nematode germline is especially amenable to examination of homolog pairing and alignment, since nuclei at all stages of meiotic prophase are present simultaneously within each gonad in an unambiguous temporal/spatial arrangement, and since DAPI-stained meiotic chromosomes can be examined in the context of well-preserved three-dimensional nuclear architecture (Dernburg *et al.* 1998). We examined the morphology of meiotic chromosomes in nuclei from the "transition zone" [where nuclei are entering meiosis and homolog pairing occurs (Dernburg *et al.* 1998)], through the pachytene region of the germline (where homologs are aligned and intimately associated,

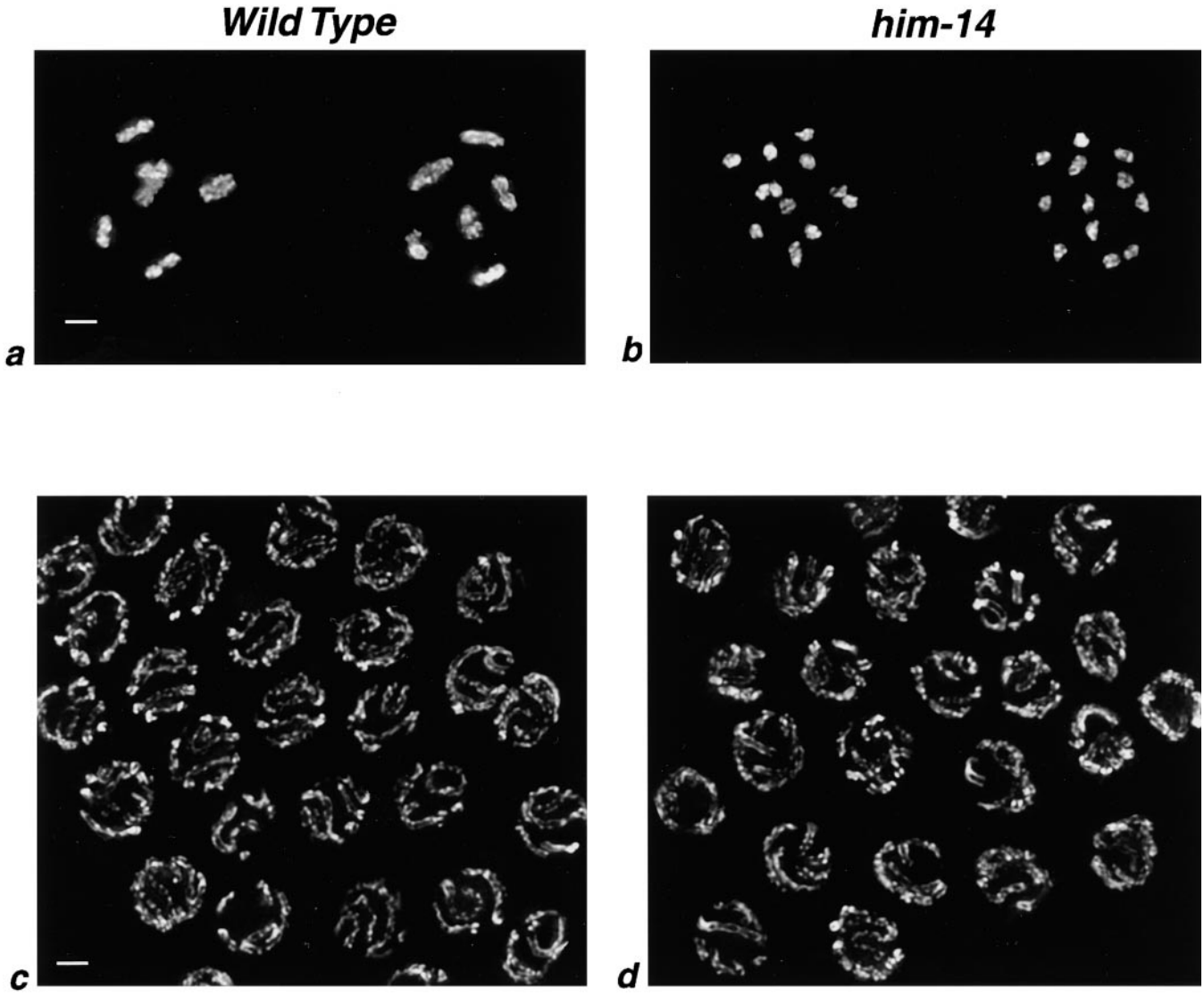


Figure 1.—Absence of chiasmata at diakinesis but normal pachytene morphology in a *him-14* mutant. (a, b) DAPI-stained oocyte nuclei at diakinesis, the final stage of meiotic prophase; each panel shows a projection of 3D data stacks through two entire nuclei. Wild-type oocytes (a) each have six bivalents, corresponding to the six pairs of homologous chromosomes attached by chiasmata. Twelve univalents are seen in *him-14(it21)* mutant oocytes (b), indicating an absence of chiasmata. (c, d) Fields of DAPI-stained germline nuclei at the pachytene stage, earlier in meiotic prophase. In both the wild-type control [c; genotype *him-14(it21)/mnC1*] and *him-14* mutant [d; genotype *him-14(it21)/him-14(it21)*] nuclei, parallel tracks of DAPI-stained cables are readily observed, corresponding to side-by-side aligned homologous chromosomes (see also Figure 2). Chromosomes are at the periphery of the nuclei at this stage; to illustrate chromosome morphology most clearly, the images shown are projections halfway through the nuclei. Scale bars, 2 μm.

or synapsed, along their entire lengths), through the diplotene/diakinesis stages (when the homologs desynapse and the chromosomes continue to condense).

The morphology of DAPI-stained meiotic chromosomes in three-dimensionally preserved germlines appeared normal in the *him-14* mutant until the diakinesis stage, when homologous chromosomes desynapsed, revealing the absence of chiasmata. Comparison of Figure 1c and 1d, shows the identical appearance of pachytene nuclei from wild-type and *him-14* mutant hermaphrodites, with their parallel DAPI-stained tracks corresponding to side-by-side aligned and synapsed homologous chromosomes (Dernburg *et al.* 1998). Further, fluorescent *in situ* hybridization (FISH) demonstrated normal intimate pairing of homologous sequences in the *him-14* mutant (Figure 2). For each probe, either a single hybridization signal or a closely spaced doublet was observed for all pachytene nuclei in both control and *him-14* hermaphrodites.

The *him-14* gene product functions during the pachytene stage of meiotic prophase: Temperature-shift experiments were carried out with the temperature-sensitive allele *him-14(it44ts)* to determine when during meiotic prophase the *him-14* gene product is required.

TABLE 1

Severe reduction in crossover frequencies in a *him-14* mutant

Interval tested	Genotype	No. of recombinants	No. of progeny scored	Map distance (cM)
dpy-3 unc-3 X	+ / *him-14*	593	1828	41
	him-14 / *him-14*	0	630	<0.2
unc-1 dpy-3 X	+ / *him-14*	62	1419	4.5
	him-14 / *him-14*	0	228	<0.4
unc-5 dpy-20 IV	+ / *him-14*	72	1323	5.6
	him-14 / *him-14*	0	157	<0.6

Recombination frequencies in *him-14* homozygotes were assessed by scoring for recombinant phenotypes among the few percent of their progeny that survive to adulthood (see materials and methods).

Young gravid adult hermaphrodites were transferred from permissive (15°) to restrictive (23°) temperature, or from restrictive to permissive temperature (Figure 3). Batches of worms were fixed and stained with DAPI at various time points following the shift, and oocyte nuclei were examined for the presence of achiasmate chromosomes. For the oocytes scored at each time point, the stage of their nuclei at the time of the shift was inferred based on germline distribution and estimated rates of progression of meiotic nuclei through the gonad (see materials and methods). In the upshift experiment, six bivalents were observed in oocyte nuclei that had been in late pachytene at the time of the shift, suggesting that these nuclei had already completed the process that requires *him-14* function prior to the end of the pachytene stage. In contrast, pachytene nuclei that had spent less time in the pachytene stage by the time of the upshift exhibited the high frequency of achiasmate chromosomes characteristic of the *him-14* mutant phenotype, indicating that *him-14* function is required during the pachytene stage. In the downshift experiment, we found that many nuclei that had already reached the pachytene stage at the restrictive temperature could be completely rescued by a shift to the permissive temperature, suggesting that the requirement for *him-14* does not begin until after the nuclei have achieved the pachytene configuration. Together these results suggest that the requirement for *him-14* function in crossing over may be restricted to the pachytene stage, corroborating the conclusion from our cytological analysis that *him-14* is not required for pairing or synapsis of homologous chromosomes and suggesting instead a more direct involvement in the recombination process itself.

***him-14* encodes a germline-specific member of the MutS family of mismatch repair proteins:** To establish the molecular identity of the *him-14* gene product, we first narrowed down the position of *him-14* on the genetic and physical maps. Three factor crosses established that *him-14* is left of *lin-26*, and very close but

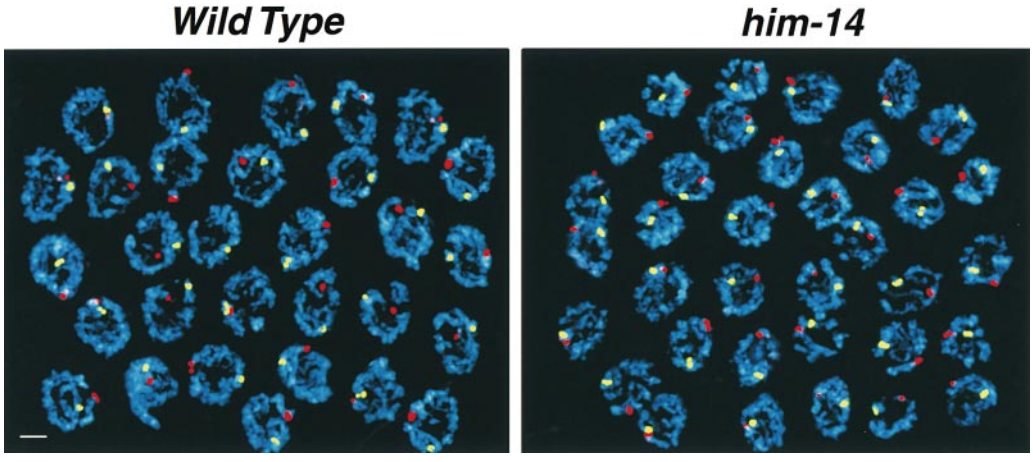


Figure 2.—Normal intimate pairing of homologous chromosomes in *him-14* pachytene nuclei. Homologous pairing was assayed by FISH in wild-type control [*him-14(it21)* / *mnC1*] and *him-14* mutant *(it21* / *it21)* hermaphrodites; images are projections through 3D data stacks encompassing whole nuclei. For both the 5S rDNA chromosome V probe (yellow) and the Y22E4 *X* chromosome probe (red), either a single hybridization signal or a closely spaced doublet is observed in each nucleus in both control and *him-14* pachytene nuclei, indicating close juxtaposition of homologous sequences. Blue, DAPI; scale bar, 2 μm.

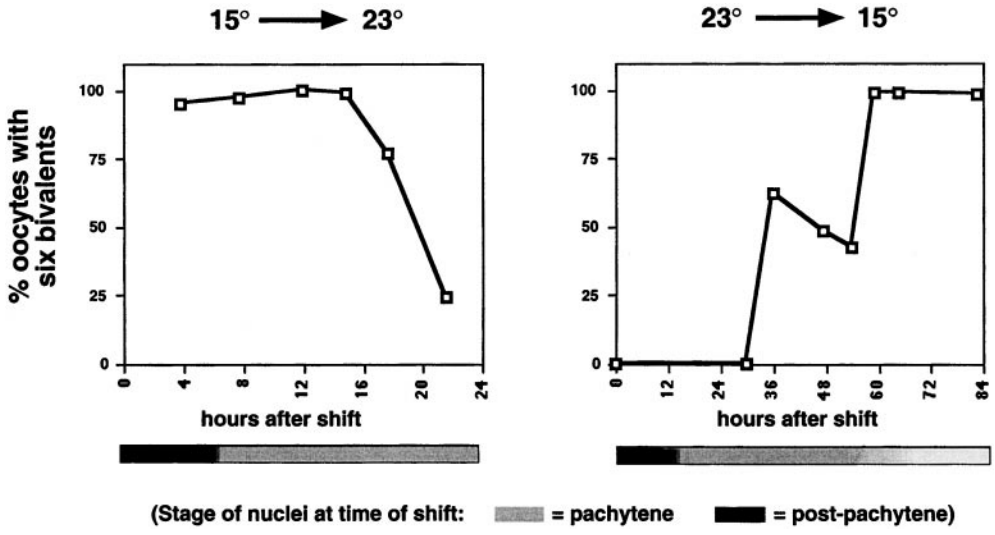


Figure 3.—*him-14* functions during the pachytene stage of meiotic prophase. Young gravid adult *him-14(it44ts)* hermaphrodites were transferred from 15° (permissive temperature) to 23° (restrictive temperature), or from 23° to 15°. Batches of worms were fixed and stained with DAPI at various times following the shift (*x* axis), and oocyte nuclei were scored for the presence of achiasmate chromosomes. The *y* axis indicates the percent of oocytes that displayed a fully wild-type phenotype (six bivalents, no achiasmate chromosomes). For oocytes scored at each time point, the grayscale bar below the graph denotes the approximate stage of meiotic prophase of their nuclei at the time of the shift (see materials and methods): black denotes postpachytene stages; medium gray denotes the pachytene stage; lighter gray denotes uncertainty about time of entry into the pachytene stage.

to the right of *unc-104* (probably within 2–3 cosmid lengths). Further, a *him-14* mutation complements chromosomal deficiencies (*mnDf30*, *mnDf88*, and *mnDf105*) with breakpoints in this region (Labouesse *et al.* 1994; this work), locating the *him-14* gene within a roughly 400-kb interval (Figure 4A).

Examination of the genome sequence of this region revealed a candidate gene that encodes a protein of the MutS family (Durbin and Thierry-Mieg 1991). This protein, ZK1127.11, is an apparent ortholog of yeast Msh4p, which promotes crossover formation during meiosis (Ross-Macdonald and Roeder 1994). Northern hybridization with a cDNA for this gene detects a 2.8-kb transcript in wild-type adult worms that is absent in worms without germlines (Figure 4C), consistent with the gene having a meiosis-specific function.

The identity of *ZK1127.11* with *him-14* was confirmed using SSCP analysis and subsequent sequencing to iden-

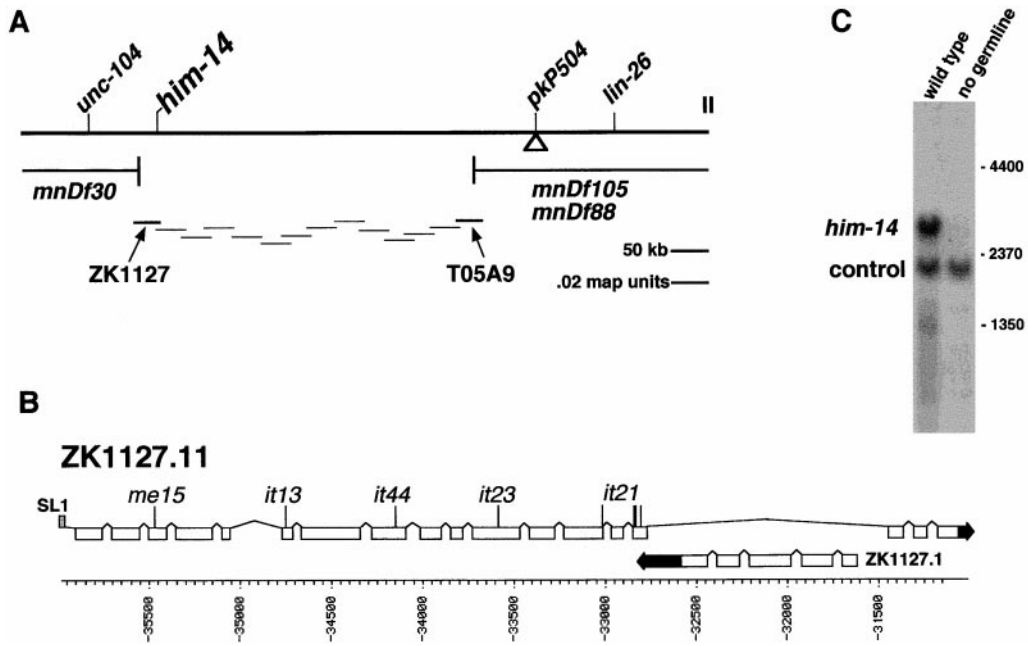
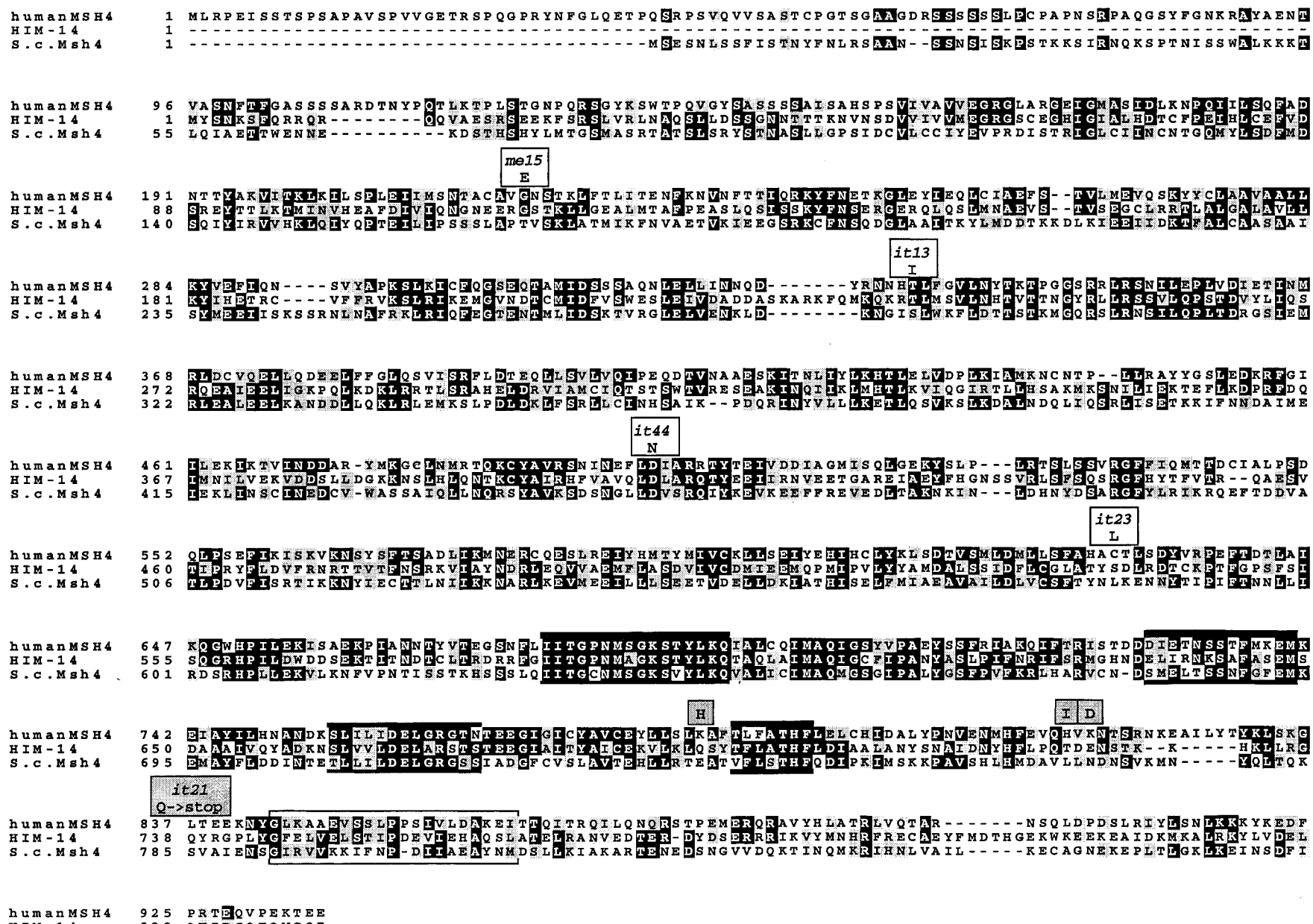


Figure 4.—*him-14* map position, gene structure, and expression. (A) The position of *him-14* relative to other genetic/physical markers (above the line), deletion breakpoints (below the line), and a contig of cosmid clones spanning the region. (B) Intron/exon structure of the ZK1127.11 gene, with positions of mutations in *him-14* mutant alleles indicated above. The SL1 *trans*-spliced leader is present at the 5′ end of the transcript; black indicates untranslated region, with the arrow indicating direction of transcription. Numerical scale corresponds to ZK1127 sequence coordinates in base-pairs. A second gene transcribed in the opposite direction is located within the 15th intron. (C) Merged images of two separate probings of a Northern blot containing RNA from young adult wild-type worms and *glp-4(bn2)* worms that lack a germline. A single 2.8-kb *him-14* transcript was detected only in worms that have a germline, while equal amounts of the control lysyl tRNA synthetase transcript were detected in both RNA samples.


humanMSH4 1 MLRPEISSTSPSAPAVSPVVGETRSPQGPRYNFGLQETPQSRPSVQVVSASTCPGTSGAAGDRSSSSSSLPCPAPNSRPAQGSYFGNKRAYAENT
HIM-14 1
S.c.Msh4 1 MSESNLSSFISTNYFNLRSAAN--SSNSISKPSTKKSIRNQKSPTNISSWALKKKT
humanMSH4 96 VASNFTFGASSSSARDTNYPQTLKTPLSTGNPQRSGYKSWTPQVGYSASSSSAISAHSPSVIVAVVEGRGLARGEIGMASIDLKNPQQILSQFAD
HIM-14 1 MYSNKSFQRRQR---------QQVAESRSEEKFSRSLVRLNAQSLLDSSGNNTTTKNVNSDVVIVVMEGRGSCEGHIGIALHDTCFPEIHLCEFVD
S.c.Msh4 55 LQIAETTWENNE----------KDSTHSHYLMTGSMASRTATSLSRYSTNASLLGPSIDCVLCCIYEVPRDISTRIGLCIINCNTGQMYLSDFMD
me15 E
humanMSH4 191 NTTVAKVITKLKILSPLEIIMSNTACAVGNSTKLFTLITENFKNVNFTTIQRKYFNETKGLEYIEQLCIAEFS--TVLMEVQSKYYCLAAVAALL
HIM-14 88 SREYTTLKTMINVHEAFDIVIQNGNEERGSTKLLGEALMTAFPEASLQSISSKYFNSERGERQLQSLMNAEVS--TVSEGCLRRTLALGALAVLL
S.c.Msh4 140 SQIYIRVVHKLQIYQPTEILIPSSSLAPTVSKLATMIKFNVAETVKIEEGSRKCFNSQDGELAAITKYLMDDTKKDLKIEEIIDKTFALCAASAAI
it13 I
humanMSH4 284 KYVEFLQN----SVYAPKSLKICFQGSEQTAMIDSSSAQNLELLINNQD-------YRNNHTLFGVLNYTKTPGGSRRLRSNILEPLVDIETINM
HIM-14 181 KYIHETRC----VFFRVKSLRIKEMGVNDTCMIDFVSWESLEIVDADDASKARKFQMKQKRTLMSVLNHTVTTNGYRLLRSSVLQPSTDVYLIQS
S.c.Msh4 235 SYMEEIISKSSRNLNAFRKLRIQFEGTENTMLLDSKTVRGLELVENKLD--------KNGISLWKFLDTTSTKMGQRSLRNSILQPLTDRGSIEM
humanMSH4 368 RLDCVQELLQDEELFFGLQSVISRFLDTEQLLSVLVQIPEQDTVNAAESKITNLIYLKHTLELVDPLKIAMKNCNTP--LLRAYYGSLEDKRFGI
HIM-14 272 RQEAIEELIGKPQLKDKLRRTLSRAHELDRVIAMCIQTSTSWTVRESEAKINQIIKLMHTLKVIQGIRTLLHSAKMKSNILIEKTEFLKDPRFDQ
S.c.Msh4 322 RLEALEELKANDDLLQKLRLEMKSLPDLDKLFSRLLCINHSAIK--PDQRINYVLLLKETLQSVKSLKDALNDQLIQSRLISETKKIFNNDAIME
it44 N
humanMSH4 461 ILEKIKTVINDDAR-YMKGCLNMRTQKCYAVRSNINEFLDIARRTYTEIVDDIAGMISQLGEKYSLP---LRTSLSSVRGFFIQMTTDCIALPSD
HIM-14 367 IMNILVEKVDDSLLDGKKNSLHLQNTKCYAIRHFVAVQLDLARQTYEEIIRNVEETGAREIAEYFHGNSSVRLSFSQSRGFHYTFVTR--QAESV
S.c.Msh4 415 IEKLINSCINEDCV-WASSAIQLLNQRSYAVKSDSNGLLDVSRQIYKEVKEEFFREVEDLTAKNKIN---LDHNYDSARGFYLRIKRQEFTDDVA
it23 L
humanMSH4 552 QLPSEFIKISKVKNSYSPTSADLIKMNERCQESLREIYHMTYMIVCKLLSEIYEHIHCLYKLSDTVSMLDMLLSFAHACTLSDYVRPEFTDTLAI
HIM-14 460 TIPRYFLDVFRNRTTVTFNSRKVIAYNDRLEQVVAEMFLASDVLVCDMIEEMQPMIPVLYYAMDALSSIDFLCGLATYSDLRDTCKPTFGPSFSI
S.c.Msh4 506 TLPDVFISRTIKKNYIECTTLNIIKKNARLKEVMEEILLLSEETVDELLDKIATHISELFMIAEAVAILDLVCSFTYNLKENNYTIPIFTNNLLI
humanMSH4 647 KQGWHPILEKISAEKPIANNTYVTEGSNFLIITGPNMSGKSTYLKQIALCQIMAQIGSYVPAEYSSFRIAKQIFTRISTDDDIETNSSTFMKEMK
HIM-14 555 SQGRHPILDWDDSEKTITNDTCLTRDRRFGIITGPNMAGKSTYLKQTAQLAIMAQIGCFIPANYASLPIFNRIFSRMGHNDELIRNKSAFASEMS
S.c.Msh4 601 RDSRHPLLEKVLKNFVPNTISSTKHSSSLQIITGCNMSGKSVYLKQVALICIMAQMGSGIPALYGSFPVFKRLHARVCN-DSMELTSSNFGFEMK
H
I D
humanMSH4 742 EIAYILHNANDKSLILIDELGRGTNTEEGIGICYAVCEYLLSLKAFTLFATHFLELCHIDALYPNVENMHFEVQHVKNTSRNKEAILYTYKLSKG
HIM-14 650 DAAAIVQYADKNSLVVLDELARSTSTEEGIAITYAICEKVLKLQSYTFLATHFLDIAALANYSNAIDNYHFLPQTDENSTK--K------HKLLRG
S.c.Msh4 695 EMAYFLDDINTETLLILDELGRGSSIADGFCVSLAVTEHLLRTEATVFLSTHFQDIPKIMSKKPAVSHLHMDAVLLNDNSVKMN-----YQLTQK
it21 Q->stop
humanMSH4 837 LTEEKNYGLKAAEVSSLPPSIVLDAKEITTQITRQILQNQRSTPEMERQRAVYHLATRLVQTAR-------NSQLDPDSLRIYLSNLKKKYKEDF
HIM-14 738 QYRGPLYGFELVELSTIPDEVIEHAQSLATELRANVEDTER-DYDSERRRIKVYMNHRFRECAEYFMDTHGEKWKEEKEAIDKMKALRKYLVDEL
S.c.Msh4 785 SVAIENSGIRVVKKIFNP-DIIAEAYNMDSLLKIAKARTENEDSNGVVDQKTINQMKRIHNLVAIL-----KECAGNEKEPLTLGKLKEINSDFI
humanMSH4 925 PRTEQVPEKTEE
HIM-14 832 AKIDSQEQMCQZ
S.c.Msh4 874 ENFEE-------

tify point mutations in five *him-14* mutant alleles. The positions of the mutations are indicated in Figure 5, which also shows an alignment of the predicted protein sequences of HIM-14, its human (Paquis-Flucklinger *et al.* 1997) and its yeast (Ross-Macdonald and Roeder 1994) orthologs. One missense mutant allele, *it13*, causes a change to isoleucine at a conserved residue that is either threonine or serine in all known eukaryotic MSH family members and in nearly all prokaryotic members as well. The temperature-sensitive allele *it44* causes a change to asparagine of an aspartic acid residue present in all MSH4 subfamily members. The *it21* allele is almost certainly null, since it contains a nonsense mutation that terminates translation upstream of both the conserved helix-turn-helix motif (thought to mediate a crucial ATP-induced conformational change that modulates mismatch binding) and a C-terminal region required for normal heterodimerization and consequent mismatch binding specificity in other members of this protein family (Alani 1996; Alani *et al.* 1997); an analogous C-terminal deletion of *S. cerevisiae* MSH2 exhibits the null phenotype *in vivo* (Alani *et al.* 1997).

Figure 4B shows the modified *him-14* gene structure determined by sequencing of cDNAs obtained both by 5′ rapid amplification of cDNA ends (RACE) and from the Kohara lab EST library (Kohara 1996). These experiments showed that the *him-14* message is SL1 *trans*-spliced, and identified three 5′ exons previously missed by the Genefinder program. The remaining intron/exon boundaries predicted by Genefinder and displayed in the ACeDB database (Durbin and Thierry-Mieg 1991) were confirmed. (The 15th intron of this gene contains a small gene transcribed in the opposite direction.)

Along with the aforementioned nonsense mutation, the *it21* mutant allele contains 15 additional base changes and several small deletions and insertions spread over a 571-bp region (coordinates 33062-32491 of ZK1127), suggesting that it arose by an unusual mechanism. A partial duplication of the *him-14* gene (termed T02G5.6) had been identified previously by the sequencing consortium at a position 10 kb from the intact *him-14* gene. This 1.1-kb duplicated DNA segment includes both exons and introns and shares >96% sequence identity with *him-14*. Nearly 30% of the *him-14* coding region is duplicated at this locus, but the partial duplicate is not expected to produce a functional protein since it lacks both the 5′ and 3′ ends and contains both a base substitution and a base deletion that create in-frame stop codons. The *it21* mutant allele was apparently generated by a gene conversion event in which this duplicated gene segment had served as the information donor, since all of the changes present in the *it21* allele match a contiguous portion of the sequence present at the T02G5.6 locus (coordinates 8946-9519 of T02G5).

Evidence that *him-14* does not function in mismatch repair: Since *him-14* encodes a member of the MutS protein family, we were led to test the possibility that *him-14* might have a role in DNA mismatch repair. Specifically, we tested whether loss of *him-14* function results in a "mutator" phenotype by comparing the frequency of spontaneous mutations conferring resistance to the drug levamisole (LevR; Lewis *et al.* 1980) between wild-type and *him-14* mutant worms. This property was assessed in the temperature-sensitive mutant *him-14(it44ts)*, since growth under permissive conditions makes it possible to generate the large populations of homozygous mutant worms required to make quantitative measurements of mutation frequencies. *him-14(it44ts)* mutant and control worm cultures synchronized at the L1 larval stage (with only two germline founder cells per worm) were shifted to restrictive temperature, and the frequency of spontaneous LevR mutations among the gametes of the shifted animals was subsequently assessed (Table 2). There was no difference in spontaneous mutation frequency between mutant and control worms, indicating that the *him-14(it44ts)* mutation does not cause any detectable defect in mismatch repair despite eliminating the *him-14* crossover-promoting activity. This suggests that *him-14*, like its ortholog *MSH4* from budding yeast (Ross-Macdonald and Roeder 1994), probably has no role in mismatch repair but rather may function specifically to promote crossing over during meiosis.

DISCUSSION

Role of *him-14* in crossover formation: We have shown here that the *C. elegans him-14* gene encodes a germline-specific member of the MutS protein family. *him-14* is required during the pachytene stage of meiotic prophase to form the crossovers that are essential for directing segregation of homologous chromosomes at the meiosis I division. HIM-14 is the nematode ortholog of the meiosis-specific Msh4p protein of *S. cerevisiae*, which together with its heterodimer partner Msh5p also functions to promote meiotic crossover formation (Ross-Macdonald and Roeder 1994; Hollingsworth *et al.* 1995; Pochart *et al.* 1997); *C. elegans* also has a *MSH5* ortholog that is similarly required for meiotic crossing

Figure 5.—Alignment of the HIM-14 predicted protein sequence with human MSH4 and yeast Msh4p sequences. Regions boxed in black correspond to the Walker homology ATP-binding motif that defines the MutS family; the white box corresponds to the predicted helix-turn-helix motif. Amino acid changes caused by *him-14* mutant alleles are indicated above the sequence; the multiple changes caused by the *it21* allele are shown in shaded boxes.

TABLE 2

Germline spontaneous mutation frequency is not increased in a *him-14* mutant

Genotype	Flask no.	Plates with LevR/total plates	$f(0)$	Mutation frequency (LevR/gamete)	Spontaneous LevR mutations/gamete
xol-1(y9)	1	0/18	1	0	
	2	4/18	0.78	1.3×10^{-5}	
	3	1/18	0.94	3.2×10^{-6}	
	4	3/18	0.83	9.5×10^{-6}	
	5	4/18	0.78	1.3×10^{-5}	
	6	3/17	0.82	1.0×10^{-6}	$8.1 \pm 5.4 \times 10^{-6}$
him-14(it44); xol-1(y9)	1	4/20	0.80	1.2×10^{-5}	
	2	3/20	0.85	8.6×10^{-6}	
	3	2/20	0.90	5.5×10^{-6}	
	4	2/20	0.90	5.5×10^{-6}	
	5	4/20	0.80	1.2×10^{-5}	
	6	6/19	0.68	2.0×10^{-5}	$1.1 \pm .54 \times 10^{-5}$

The frequency of spontaneous germline mutations conferring levamisole resistance (LevR) was assessed in *him-14* mutant worms [*him-14(it44); xol-1(y9)*] and control [*xol-1(y9)*] worms. For both genotypes, six independent liquid cultures, each containing $\sim 3 \times 10^6$ synchronized L1 larvae, were shifted to the restrictive temperature (23°). The frequency of LevR mutations among the gametes of these shifted animals was measured by plating multiple aliquots of their F_1 progeny and testing for the segregation of resistant worms in the subsequent generation, as described in materials and methods. Mutation frequencies for each independent culture were calculated as: [*m*, the mean number of spontaneous mutations per plate]/19,000 [= number of haploid genomes represented per plate]. *m* was calculated using the Poisson equation $f(0) = e^{-m}$, where $f(0)$ = the fraction of the plates from a given culture that did not contain a LevR mutant.

over (Durbin and Thierry-Mieg 1991; K. Kelly and A. M. Villeneuve, unpublished results). Both HIM-14 and Msh4p appear to function exclusively in meiotic recombination, having no apparent roles in the mismatch repair processes that initially defined the MutS family. How have these proteins harnessed conserved features of the MutS proteins and directed them to promote crossover recombination rather than mismatch repair?

Recent studies of eukaryotic MutS family members that function in mismatch repair have provided a new context for thinking about how the HIM-14/MSH-5 heterodimer might function in crossover formation. Two groups have now clearly demonstrated that following mismatch recognition by the human MSH2/MSH6 heterodimer, binding of ATP alters the affinity of MSH2/MSH6 for the mismatch and stimulates its movement along the DNA backbone (Blackwell *et al.* 1998; Gradia *et al.* 1999). Although the two groups propose conflicting models for the mechanism of this movement (ATP-dependent translocation *vs.* sliding clamp), both invoke a conformational transition that results in travel of the MSH2/MSH6 complex along a DNA duplex, away from the original site of mismatch recognition.

By analogy to the binding of MSH2/MSH6 and MSH2/MSH3 to base mismatches and small insertional mismatches, it has been suggested previously that MSH4/MSH5 might promote crossover formation through recognition of and binding to other types of distortions in DNA duplex structure found in predicted recombination intermediates, particularly Holliday junctions (Ross-Macdonald and Roeder 1994; Hollingsworth et al. 1995). How this recognition and binding might then translate into promoting resolution via a crossover pathway was not clear. The sliding/translocation paradigms proposed for MSH2/MSH6 now provide a concrete framework for thinking about how binding to Holliday junctions (or their precursors) could directly promote crossover resolution. Several currently favored models for resolution of recombination intermediates to yield noncrossover products require branch migration of either one or both Holliday junctions present in a double Holliday junction intermediate (Gilbertson and Stahl 1996; Stahl 1996). For example, one class of model proposes that noncrossover prod-

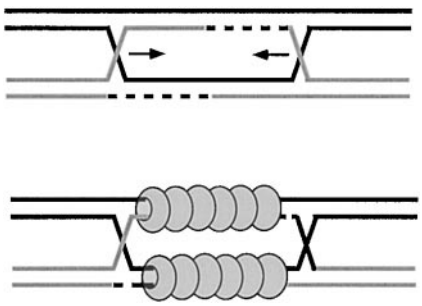


Figure 6.—Model: accumulation of HIM-14/MSH-5 heterodimers promotes crossover resolution of double Holliday junction recombination intermediates by interfering with branch migration. See text.

ucts arise when branch migration leads to convergence of the two Holliday junctions (Figure 6), with the resulting strand intertwinings resolved by topoisomerase action. We suggest a model in which MSH4/MSH5 heterodimers are converted into a sliding- or translocation-competent configuration following recognition of junctions or branched-molecule junction precursors. As a consequence, heterodimers could then accumulate between the two Holliday junctions (Figure 6), thereby interfering with branch migration and constraining the intermediate to undergo resolution via a pathway that does not require branch migration, *i.e.*, the crossover pathway.

Differential reliance on a conserved recombination pathway: Despite conservation of the crossover-promoting activity of the HIM-14/Msh4p proteins, nematodes and yeast differ substantially in the extent to which they depend on this conserved pathway for crossover formation. Since worms rely almost exclusively on this pathway to generate crossovers, eliminating HIM-14 eliminates nearly all crossovers, producing disastrous consequences for chromosome segregation and resulting in recovery of very few viable progeny. In contrast, yeast mutants that lack Msh4p, Msh5p, or both, can still generate 30–50% of the normal number of crossovers (Ross-Macdonald and Roeder 1994; Hollingsworth *et al.* 1995); thus most yeast chromosomes will still enjoy at least one crossover in a *msh4* mutant, which consequently exhibits a relatively modest 45–60% decrease in spore viability (Ross-Macdonald and Roeder 1994).

What might account for the substantial difference in the extent to which nematodes and yeast rely on the HIM-14/MSH4 pathway for crossover formation? One possibility is that the role of HIM-14/Msh4p is to commit a subset of recombination intermediates to a conformation that facilitates resolution via the crossover pathway. According to this scenario, recombination intermediates in yeast would have a high probability of forming or maintaining a crossover-competent conformation even in the absence of this Msh4p stabilizing interaction, whereas in the nematode, the HIM-14 stabilizing interaction is crucial for establishing or maintaining a crossover-competent conformation. Structural differences between nematode and yeast pachytene chromosomes might potentially contribute to a differential stability of recombination intermediates. *C. elegans* pachytene chromosomes achieve a sixfold greater degree of chromosome compaction than do yeast pachytene chromosomes (Byers and Goetsch 1975; Goldstein and Slaton 1982; The Yeast Genome Directory 1997; The *C. elegans* Sequencing Consortium 1998), raising the possibility that the DNA molecules themselves may be under different physical constraints as a consequence of this difference in chromosome compaction.

An alternative model is that there may be two distinct classes of crossovers in yeast, and only one of these two classes clearly predominates in nematodes. It was shown recently that both nematodes and Drosophila differ dramatically from yeast with respect to the relationship between recombination initiation and homologous synapsis of chromosomes during meiosis. A substantial body of evidence has led to the conclusion that formation of the synaptonemal complex (SC) between homologous chromosomes in yeast is dependent upon early events in recombination (Kleckner 1996; Roeder 1997), and further that both synapsis and homologous chromosome pairing are dependent on the recombination-initiating enzyme Spo11p as well as other proteins required for initiation (Weiner and Kleckner 1994; Keeney *et al.* 1997). In contrast, although recombination initiation in worms (and flies) occurs by a *spo-11*-dependent double-strand break mechanism that is conserved with yeast, intimate pairing and synapsis in *C. elegans* and Drosophila are *independent* of recombination initiation and *spo-11* (Dernburg *et al.* 1998; McKim *et al.* 1998; McKim and Hayashi-Hagihara 1998). McKim *et al.* have argued that instead large-scale synapsis may in fact be a prerequisite for recombination initiation in Drosophila. This difference in the relationship between recombination and synapsis has led us to suggest that the two distinct classes of crossovers that occur in yeast may be (1) crossovers that correspond to recombination events that occur at sites of initiation of synapsis (and are the triggers for synapsis), or (2) crossovers that correspond to recombination events in which intermediates form in the context of already synapsed chromosomes, and in fact depend on synapsis to be resolved as crossovers. According to this model, the second type of crossovers, those that occur in the context of and depend on SC, would predominate in *C. elegans*.

The existence of the two proposed classes of crossover events in yeast has extensive experimental support. First, null mutations in several other yeast genes cause reductions in crossing over of a magnitude similar to that seen in *msh4* and *msh5* mutants (Sym *et al.* 1993; Sym and Roeder 1994; Hunter and Borts 1997; Chua and Roeder 1998). Notable in this class are *zip1* and *zip2* mutants, which exhibit specific defects in chromosome synapsis (Sym *et al.* 1993; Sym and Roeder 1994; Chua and Roeder 1998). *ZIP1* encodes a structural component of the central region of the synaptonemal complex, and the *ZIP2* gene product is required for polymerization of Zip1p. In the absence of either of these two gene products, homologous chromosomes with cytologically differentiated axial elements are aligned side by side, but are only intimately connected at a few sites, called axial associations, that are thought to correspond to sites of recombination initiation (Sym *et al.* 1993; Rockmill *et al.* 1995; Roeder 1997; Chua and Roeder 1998). Interestingly, the number of axial associations observed in a *zip1* mutant is approximately half the total number of crossovers that occurs during meiosis in wild type. Moreover, epistasis experiments indicate that a *zip1* null mutation eliminates the same subset of crossovers that

is dependent on Msh4p/Msh5p (P. Ross-Macdonald and G. S. Roeder, personal communication). A parsimonious explanation is that only the first, SC-*independent* class of crossovers occurs in the absence of these gene products. It is notable that both *zip1* and *msh4* mutations have been found to eliminate crossover interference (Sym and Roeder 1994; Roeder 1997). If our model is correct, the implication is that the first class, the SC-independent recombination events that serve as synapsis initiators, may not be subject to crossover interference.

Conclusion: A persistent theme of differential reliance on conserved recombination components has emerged not only from the present study of *him-14*, but also through the previous report on *C. elegans spo-11* (Dernburg *et al.* 1998) and ongoing work on other genes encoding components of the nematode recombination machinery (K. Kelly, G. Chin and A. M. Villeneuve, unpublished results). These studies on meiotic recombination in the nematode have helped build a compelling case that the basic DNA enzymology underlying many events in meiotic recombination is widely conserved. At the same time, they have graphically illustrated that eliminating conserved components or pathways can have very different consequences for diverged organisms. These findings highlight the importance of studying fundamental biological processes in more than one experimental system, and make it clear that the *C. elegans* system has much to contribute to our understanding of chromosome behavior during meiosis.

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