1   **The conserved ATPase PCH-2 controls the number and distribution of crossovers by**

2   **antagonizing crossover formation in *C. elegans***

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16 **Abstract**

17

18 Meiotic crossover recombination is essential for both accurate chromosome segregation and the

19 generation of new haplotypes for natural selection to act upon. While the conserved role of the

20 ATPase, PCH-2, during meiotic prophase has been enigmatic, a universal phenotype that is observed

21 when *pch-2* or its orthologs are mutated is a change in the number and distribution of meiotic

22 crossovers. Here, we show that PCH-2 controls the number and distribution of crossovers by

23 antagonizing crossover formation. This antagonism produces different effects at different stages of

24 meiotic prophase: early in meiotic prophase, PCH-2 prevents double strand breaks from becoming

25 crossovers, limiting crossovers at sites of initial DSB formation and homolog interactions. Later in

26 meiotic prophase, PCH-2 winnows the number of crossover-eligible intermediates, contributing to the

27 reinforcement of crossover-eligible intermediates, designation of crossovers and ultimately, crossover

28 assurance. We also demonstrate that PCH-2 accomplishes this regulation through the meiotic

29 HORMAD, HIM-3. Our data strongly support a model in which PCH-2's conserved role is to remodel

30 meiotic HORMADs throughout meiotic prophase to destabilize crossover-eligible precursors,

31 coordinate meiotic recombination with synapsis, and contribute to the progressive implementation of

32 meiotic recombination, guaranteeing crossover control.

Introduction

Meiosis is a specialized type of cell division that reduces chromosome number by half, resulting in the production of four genetically diverse haploid gametes, so that fertilization during sexual reproduction restores diploidy. This process occurs in two stages: meiosis I, in which homologous chromosomes are partitioned, and meiosis II, in which sister chromatid are segregated. The regulation of meiosis is crucial for ensuring that both genetic recombination and chromosome segregation occur accurately. During prophase I, homologous chromosomes pair and this pairing is stabilized through a process called synapsis, in which a protein structure called the synaptonemal complex (SC) holds homologs together. This close association between homologs during synapsis facilitates crossover formation, the process in which double strand breaks (DSBs) are deliberately introduced into the genome, repaired by meiosis-specific mechanisms that exchange DNA between homologous chromosomes and generate the chiasmata, or linkage, that promotes accurate meiotic chromosome segregation. Therefore, disruptions in pairing, synapsis, or recombination prevent the formation of chiasmata and can lead to meiotic errors such as nondisjunction, resulting in aneuploid gametes. Errors in human meiosis are associated with birth defects, such as Down and Turner syndromes, infertility and miscarriages, underscoring the importance of understanding meiosis to human health (Hassold & Hunt, 2001).

In addition to the fundamental role that recombination plays in ensuring accurate chromosome segregation, crossover recombination accomplishes another important function: it generates new haplotypes for natural selection to act upon to drive evolution. Thus, to assure a random assortment of alleles on a population level, the distribution of crossovers may be as tightly regulated as their number. The significance of controlling both crossover number and distribution is clearly illustrated by the existence of mechanisms such as crossover assurance, in which every pair of homologous chromosomes gets at least one crossover; crossover homeostasis, in which the number of crossovers remains relatively invariant even if the number of recombination precursors change; and crossover

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60   interference, in which the presence of a crossover inhibits the formation of a crossover nearby (Gray &

61   Cohen, 2016). DSBs typically vastly outnumber crossovers in most organisms and are introduced

62   gradually throughout early prophase. Therefore, to accomplish this precise level of control, meiotic

63   crossover recombination and the decision about which DSBs become crossover-eligible

64   intermediates, and eventually crossovers, is implemented progressively throughout meiotic prophase

65   (Cole et al., 2012; Joshi et al., 2015; Morgan et al., 2021; Yokoo et al., 2012).

66

67   PCH-2, also known as TRIP13 in mammals, is an evolutionarily ancient AAA-ATPase that plays a

68   significant role in regulating meiosis across different organisms, including *M. musculus* (mice), *S.*

69   *cerevisiae* (budding yeast), *D. melanogaster* (fruit flies), and *C. elegans* (worms) (Bhalla, 2023). PCH-

70   2 and its orthologs structurally remodel a family of proteins with HORMA domains (HORMADs) to

71   control their function (Gu et al., 2022). HORMADs participate in a variety of signaling events and can

72   exist in at least three structurally distinct conformations: a "closed" conformation, which they adopt

73   when they bind a short peptide sequence in their own protein sequence or another protein (also called

74   a closure motif) and their C-terminus wraps around this motif to stabilize the interaction; an "open"

75   conformation when unbound, in which their C-terminus is discretely tucked against the HORMA

76   domain; and an "extended" conformation, which is an intermediate between the two (Gu et al., 2022).

77   PCH-2 and its orthologs convert the closed version of HORMADs to the open or extended versions,

78   playing an important role in recycling HORMADs during signaling. During meiosis, closed versions of

79   meiotic HORMADs assemble on chromosomes to form meiotic chromosome axes, which are essential

80   for pairing, synapsis and recombination between homologous chromosomes (Kim et al., 2014). The

81   remodeling of meiotic HORMADs by PCH-2 to an "open" or "extended" conformation is thought to

82   reduce the levels of HORMADs on chromosomes (Börner et al., 2008; Cuacos et al., 2021; Lambing

83   et al., 2015; Wojtasz et al., 2009) and/or increase their dynamic association and dissociation (Russo et

84   al., 2023), modulating and coordinating homolog pairing, synapsis and recombination during

85   prophase.

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87   The conserved role of the PCH-2/HORMAD module in meiosis has been difficult to characterize, in

88   part, we have argued, because of the evolutionary innovation that is an inherent aspect of sexual

89   reproduction (Bhalla, 2023). Moreover, in some systems, such as budding yeast and plants (Herruzo

90   et al., 2021; Yang et al., 2020), PCH-2 orthologs not only remodel meiotic HORMADs on meiotic

91   chromosomes but also perform this function in the cytoplasm to make meiotic HORMADs available for

92   their role(s) in meiotic nuclei. This dual role can complicate functional analyses, particularly in *pch-2*

93   null mutants. However, all meiotic systems exhibit defects in the number and distribution of crossovers

94   when PCH-2 function is abrogated by mutation (Deshong et al., 2014; Joshi et al., 2009; Joyce &

95   McKim, 2009; Lambing et al., 2015; Roig et al., 2010; Zanders & Alani, 2009). Unfortunately, the lack

96   of a clear pattern when analyzing these defects in recombination in *pch-2* mutants has contributed to

97   an inability to develop a unified, integrated model of PCH-2 function in the field.

98

99   In *C. elegans*, meiotic HORMADs localize to meiotic chromosomes independently of PCH-2 (Deshong

100   et al., 2014). Here, we exploit this system to show that PCH-2 is required to control the number and

101   distribution of crossovers by antagonizing the formation of crossovers. This antagonism produces

102   different consequences depending on the stage of meiotic prophase. In early meiotic prophase, PCH-

103   2 inhibits DSBs from becoming crossovers, ensuring that they are more widely distributed than sites of

104   initial DSB formation and/or homolog interactions. Later in meiotic prophase, PCH-2 is responsible for

105   winnowing the numbers of crossover-eligible intermediates on synapsed chromosomes, contributing

106   to the reinforcement of crossover-eligible intermediates, designation of crossovers and ultimately,

107   crossover assurance. Genetic analysis demonstrates that PCH-2's regulation of crossover-eligible

108   intermediates is through one of three essential meiotic HORMADs, HIM-3. Finally, we link PCH-2's

109   effect on early DSBs in early meiotic prophase to cell cycle stage, demonstrating that both limit early

110   DSBs from becoming crossovers. We propose that PCH-2's remodeling of HIM-3 on meiotic

111   chromosomes destabilizes crossover-eligible intermediates throughout meiotic prophase, contributing

112   to the progressive implementation of meiotic recombination to control the number and distribution of

113   crossovers and accomplish crossover control.

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Results

PCH-2 controls the number and distribution of crossovers in similar patterns on multiple chromosomes

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We had previously shown that loss of PCH-2 reduced the frequency of double crossovers and genetic length of both an autosome (chromosome III) and the X chromosome, albeit not uniformly among genetic intervals (Deshong et al., 2014). To determine whether a more obvious pattern could be observed, we expanded our analysis by monitoring recombination genetically in both wildtype and *pch-2* mutant animals using five single nucleotide polymorphisms (SNPs) that spanned 95% of Chromosomes, I, III, IV and the X chromosome (Figure 1). We excluded Chromosome II from our analysis because of the potential difficulty combining Hawaiian SNPs with the *pch-2* mutation, which is linked to Chromosome II, and Chromosome V because of our use of the *bcIs39* transgene to identify cross progeny, which may disrupt recombination on that chromosome.

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In wildtype animals, we observed multiple double crossovers, ranging from 1-13 depending on the chromosome. In a majority of these double crossovers (69%), one crossover was at the end of the chromosome where pairing and synapsis initiate, also called the Pairing Center (PC) (MacQueen et al., 2005), suggesting some relationship between where chromosomes might make initial contacts and the likelihood of double crossovers. On all chromosomes analyzed, we observed no double crossovers in *pch-2* mutants and this difference was statistically significant for Chromosomes I, III and X. Moreover, there was a striking shift of crossovers to the PC end of all four chromosomes tested. This shift in the distribution of crossovers to the PC ends of chromosomes was generally accompanied by a reduction in crossovers in the center of chromosomes. In *C. elegans*, the center of chromosomes are where double strand breaks are less numerous (Nadarajan et al., 2021; Yu et al., 2016) and where genes are more abundant. In the case of the X chromosome and chromosome III, recombination at the non-PC end was also reduced and appeared to more closely resemble the physical map at this

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141   end of these chromosomes. Thus, PCH-2 ensures a wider distribution of crossovers across

142   chromosomes, away from regions that are more likely to undergo early homolog interactions (PC

143   ends) and with more double strand breaks (both the PC and non-PC ends of chromosomes), and

144   towards the center of chromosomes, where double strand breaks are less abundant.

145

146   **PCH-2 prevents exogenous DSBs early in meiotic prophase from becoming crossovers**

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148   We previously showed that PCH-2 promotes crossover formation and crossover assurance through its

149   regulation of the meiotic HORMAD, HIM-3, in *C. elegans* (Russo et al., 2023). Moreover, this

150   promoting role seems linked to its localization to the synaptonemal complex (Patel et al., 2023).

151   However, the observed shift in the distribution of crossovers in *pch-2* mutants suggests that PCH-2

152   may also play a role in inhibiting crossovers and that this role may be occurring in early meiotic

153   prophase, when chromosomes are undergoing initial homolog interactions. Previous data from our lab

154   had suggested this possibility, but experimental caveats limited our ability to properly interpret these

155   experiments (Deshong et al., 2014).

156

157   To explicitly test this possibility, we took advantage of both the spatiotemporal organization of meiotic

158   nuclei in the *C. elegans* germline, and the observation that nuclei travel in an assembly line process at

159   a stereotypical pace to late pachytene (Jaramillo-Lambert et al., 2007), where we can cytologically

160   assess crossover formation by staining for the essential crossover factor, COSA-1 (Yokoo et al.,

161   2012). We performed irradiation experiments to introduce exogenous DSBs at different timepoints

162   during meiotic prophase and analyzed PCH-2's role in crossover formation (Figure 2A).

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164   In this experiment, we tested the effects of ionizing radiation at two distinct time points: 8 hours after

165   irradiation, when we can monitor crossover formation in meiotic nuclei that received exogenous DSBs

166   in mid-pachytene, and 24 hours after irradiation, when we monitor crossover formation in meiotic

167 nuclei that received exogenous DSBs in leptotene/zygotene, also known as the transition zone in *C.*

168 *elegans*. In order to detect crossovers, control and *pch-2* mutants germlines were stained for

169 GFP::COSA-1, which allowed us to visualize crossovers, and DAPI, which allowed us to visualize

170 DNA. At 8 hours after irradiation, we observed a significant increase in nuclei containing less than 6

171 COSA-1 nuclei in *pch-2* mutants (26%), when compared to control (9%) germlines (Figure 2B, 2C).

172 We did not observe a significant number of nuclei with greater than 6 COSA-1 foci in either

173 backgrounds. This observation is consistent with our and others' previous findings: exogenous DSBs

174 in mid-pachytene do not affect the number of crossovers in wildtype animals (Yokoo et al., 2012) and

175 loss of PCH-2 leads to a decrease in crossover formation (Deshong et al., 2014).

176

177 Next, we examined crossover formation 24 hours post-irradiation (Figure 2D, 2E). For this time point,

178 we also saw a significant increase in nuclei containing less than 6 COSA-1 foci (control: 13%, *pch-2*:

179 26%), similar to the 8 hour time point. However, in contrast to the 8 hour time point, we also observed

180 a significant increase in nuclei with more than 6 COSA-1 foci in *pch-2* mutants (wildtype: 2%, *pch-2*:

181 13%), indicating that loss of PCH-2 leads to increased crossover formation when nuclei in the

182 transition zone get more DSBs. In other words, PCH-2 inhibits exogenous DSBs in early meiotic

183 prophase from becoming crossovers, consistent with our analysis of recombination on multiple

184 chromosomes.

185

186 **PCH-2 prevents SPO-11-induced DSBs from becoming crossovers in early meiotic prophase**

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188 Our previous experiment indicated that PCH-2 is preventing exogenous DSBs in early meiotic

189 prophase from becoming crossovers (Figure 2). To test whether this was also the case for

190 programmed meiotic DSB formation, we used the auxin-inducible degradation (AID) system to remove

191 the enzyme that is responsible for programmed meiotic DSBs, SPO-11 (*spo-11::AID::3XFLAG*)

192 (Dernburg et al., 1998; Keeney et al., 1997). Since meiotic nuclei travel through the germline at about

193 one cell per row per hour (Jaramillo-Lambert et al., 2007), we took advantage of this precise

194   spatiotemporal resolution to test five different timepoints to determine if acute depletion of SPO-11

195   leads to any changes in crossover formation between control animals and *pch-2* mutants (Figure 3A).

196   We assayed crossover formation by analyzing both the number of GFP::COSA-1 foci in late

197   pachytene and the presence of bivalents in diakinesis, which indicates the successful formation of

198   chiasmata between homologous chromosomes. Meiotic nuclei travel from late pachytene to diakinesis

199   in approximately 12 hours (Deshong et al., 2014).

200

201   When we treated both control and *pch-2* mutant worms for 36 hours with auxin, we observed a

202   complete loss of GFP::COSA-1 foci (Figure 3C). When these animals were treated with auxin for 48

203   hours, to allow nuclei without SPO-11 an additional 12 hours to reach diakinesis, we observed 12

204   DAPI stained bodies, or univalents, which are the 6 homolog pairs that have not formed chiasmata

205   (Figure 3E). Thus, we can reliably remove SPO-11 from meiotic nuclei throughout the germline and

206   prevent crossover formation.

207

208   Having established that we can reliably remove SPO-11 with the AID system, we performed the same

209   experiment at additional timepoints that remove SPO-11 at different stages of early meiotic prophase,

210   monitoring crossover formation both at late pachytene and 12 hours later in diakinesis: 24 hours to

211   monitor GFP::COSA-1 foci and 36 hours to monitor bivalent formation; 22 (GFP::COSA-1 foci) and 34

212   (bivalent formation) hours; 20 (GFP::COSA-1 foci) and 32 hours (bivalent formation); and 18

213   (GFP::COSA-1 foci) and 30 hours (bivalent formation). We also performed a control experiment,

214   treating worms with ethanol for the 24 and 36 hour timepoints to ensure that this tagged version of

215   SPO-11 was fully functional for crossover formation (Figure 3C, 3D).

216

217   At the 24 hour time point, when meiotic nuclei in the transition zone do not receive SPO-11-induced

218   DSBs, we observe a range of GFP::COSA-1 foci in late pachytene in both control animals and *pch-2*

219   mutants with a majority of nuclei having 0 or 1 focus (control and *pch-2* averages: 1.8 GFP::COSA-1

220   foci), indicating a loss of crossover formation, albeit with some heterogeneity. This was confirmed

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221   when we monitored bivalent formation at the 36 hour timepoint in both strains. This heterogeneity

222   likely reflects that it takes 1-4 hours to significantly affect DSB formation through acute degradation of

223   SPO-11 at this stage of meiosis in *C. elegans* (Hicks et al., 2022). In addition, there was no statistically

224   significant difference in the number of GFP::COSA-1 foci between control animals and *pch-2* mutants.

225   However, we did observe a slight, statistically significant difference in the number of DAPI stained

226   bodies between control animals (average number of DAPI stained bodies: 10.6) and *pch-2* mutants

227   (average number of DAPI stained bodies: 9.6), suggesting that even at this very early time point, we

228   can detect a role for PCH-2 in preventing early SPO-11-induced DSBs from becoming crossovers

229   (Figure 3D, 3E).

230

231   We continued to monitor GFP::COSA-1 and bivalent formation at successive timepoints. At the 22

232   hours and 34 hour timepoints, where SPO-11 depletion begins later in the transition zone, we saw an

233   increase in the number of GFP::COSA-1 foci and a reduction in the number of DAPI stained bodies,

234   suggesting that more DSBs are becoming crossovers. This trend continued at the 20 hour and 32 hour

235   timepoints, eventually reaching wildtype numbers for GFP::COSA-1 and DAPI stained bodies by the

236   18 hour and 30 hour timepoints, respectively.

237

238   At the 22 hour and 36 hour timepoints, we did not detect any statistically significant difference in the

239   number of GFP::COSA-1 foci or DAPI stained bodies between control animals and *pch-2* mutants,

240   even when we analyzed the number of nuclei that had greater than 6 GFP::COSA-1 foci. In contrast,

241   while the number of GFP::COSA-1 foci was not statistically significantly different between control

242   animals and *pch-2* mutants at the 20 hour time point, the number of nuclei with greater than 6

243   GFP::COSA-1 foci was significantly higher in *pch-2* mutants (control: 3%, *pch-2*: 10%, p value =

244   0.009, Fischer's Exact Test), similar to our analysis of GFP::COSA-1 24 hours after irradiation. Even

245   more strikingly, the number of DAPI stained bodies was significantly lower in *pch-2* animals than

246   control animals at the corresponding 32 hour timepoint, indicating that more homolog pairs had

247   successfully formed chiasmata in *pch-2* mutants. The inconsistency between the average number of

248 GFP::COSA-1 foci and DAPI stained bodies at this time point can be explained by our ability to more

249 reliably detect *pch-2*'s loss of crossover assurance when quantifying GFP::COSA-1 foci than when

250 analyzing bivalent formation (Deshong et al., 2014; Russo et al., 2023). No difference in GFP::COSA-

251 1 foci or bivalent formation was observed at the 18 hour and 30 hours timepoints, respectively. These

252 data indicate that during a narrow time frame of early meiotic prophase, likely during

253 leptotene/zygotene given the delay in limiting DSB formation with auxin-induced degradation of SPO-

254 11, PCH-2 prevents SPO-11-induced DSBs from becoming crossovers and chiasmata.

255

256 **PCH-2 is required for timely loading and removal of MSH-5 on meiotic chromosomes through**

257 **its regulation of HIM-3**

258

259 After DSB formation, a subset of DSBs are repaired through a pro-crossover pathway and eventually

260 winnowed to a stereotypical number of crossovers. In *C. elegans*, this can be visualized by the loading

261 of the pro-crossover factor MSH-5, a component of the meiosis-specific MutS γ complex that

262 stabilizes crossover-specific DNA repair intermediates called joint molecules (Janisiw et al., 2018;

263 Kelly et al., 2000; Snowden et al., 2004). GFP::MSH-5 begins to form a few foci in the transition zone,

264 becoming more numerous in early pachytene before decreasing in number in mid pachytene to

265 ultimately colocalize with COSA-1 marked sites in late pachytene (Janisiw et al., 2018; Woglar &

266 Villeneuve, 2018; see control in Figure 4A, 4B). The mechanism through which MSH-5 functions with

267 the SC and other meiosis factors to ultimately form crossovers has not been established but may

268 involve phosphorylation by cyclin-dependent kinases (Haversat et al., 2022; Zhang et al., 2021).

269

270 Given that we have shown that PCH-2 prevents early DSBs from becoming crossovers (Figures 2 and

271 3), we tested whether we could cytologically detect this inhibition at the level of MSH-5 behavior in

272 control animals and *pch-2* mutants. We generated wildtype and *pch-2* mutants with GFP::MSH-5 and

273 quantified the average number of GFP::MSH-5 foci per row of nuclei from the transition zone to late

274   pachytene (Figure 4A, 4B). We observed a statistically significant increase in the average number of

275   GFP::MSH-5 foci per row in the transition zone in *pch-2* mutants (Figure 4A, 4B, p value < 0.0001,

276   Student's t-test), indicating that PCH-2 typically limits MSH-5 loading at this early stage of meiotic

277   prophase. The average number of GFP::MSH-5 foci per row were similar in number in early pachytene

278   in both backgrounds. However, we also observed a substantial increase in the average number of

279   GFP::MSH-5 foci per row in *pch-2* mutants in the mid and late pachytene regions (Figure 4A, 4B),

280   indicating that PCH-2 also promotes the turnover or maturation of GFP::MSH-5 foci at these later

281   stages of meiotic prophase. Thus, PCH-2 prevents the loading of the crossover-promoting factor,

282   MSH-5, during early meiotic prophase, consistent with both our genetic analysis of recombination

283   (Figure 1) and its role in preventing early DSBs from becoming crossovers (Figures 2 and 3).

284   However, PCH-2 also appears to limit the number of crossover-eligible intermediates in mid and late

285   pachytene, an unexpected observation given that the most severe recombination defect in *pch-2*

286   mutants is the loss of crossover assurance (Deshong et al., 2014).

287

288   We previously showed that PCH-2 genetically interacts with meiotic HORMADs to effectively control

289   different aspects of meiosis: PCH-2 regulates pairing and synapsis through its regulation of HTP-3 and

290   crossover recombination through its regulation of HIM-3 (Russo et al., 2023). To determine which

291   meiotic HORMAD PCH-2 might be regulating to affect GFP::MSH-5's loading and removal on meiotic

292   chromosomes, we constructed *gfp::msh-5;htp-3^{H96Y}, gfp::msh-5;pch-2;htp-3^{H96Y}, gfp::msh-5;him-3^{R93Y}*

293   and *gfp::msh-5;pch-2;him-3^{R93Y}* mutants and quantified GFP::MSH-5 foci throughout the germline.

294   Both *htp-3^{H96Y}* and *him-3^{R93Y}* mutants showed a drastic increase in the average number of GFP::MSH-

295   5 per row of nuclei throughout the germline (Figures 4C and 4D, Supplemental Figure 1). For

296   example, compared to control germlines that peaked at approximately an average of 20 MSH-5 foci

297   per row in early pachytene and then decreased in mid-pachytene, *him-3^{R93Y}* germlines had closer to

298   an average of 30 GFP::MSH-5 foci per row in early pachytene. Moreover, the average number of

299   GFP::MSH-5 foci per row increased further in mid and late pachytene, achieving peaks closer to an

300   average of 35 foci (Figure 4D). These very high numbers of GFP::MSH-5 were striking but do not

301   produce an increased number of crossovers in *htp-3*[H96Y] or *him-3*[R93Y] single mutants (Russo et al.,

302   2023), raising the possibility that not all of them reflect functional, crossover-specific intermediates,

303   similar to what has been observed in the absence of synapsis (Woglar & Villeneuve, 2018). These

304   data also suggest that these mutations, which disrupt the ability of meiotic HORMADs to adopt the

305   "closed" conformation (Russo et al., 2023), affect the behavior of GFP::MSH-5 on meiotic

306   chromosomes.

307

308   Quantification of GFP::MSH-5 foci in *pch-2;htp-3*[H93Y] double mutants was similar to *htp-3*[H93Y] single

309   mutants, suggesting that PCH-2's effect on the behavior of GFP::MSH-5 foci was not through its

310   regulation of HTP-3 (Supplemental Figure 1). In stark contrast to *pch-2;htp-3*[H93Y] double mutants, the

311   *pch-2*;*him-3*[R93Y] double mutant showed a dramatic decrease in the overall average of MSH-5 foci per

312   row throughout the germline (Figure 4C, 4D). These averages fell between the averages in control and

313   *pch-2* mutant animals, particularly in the transition zone and late pachytene (Figure 4D), consistent

314   with our previous report that this double mutant has fewer defects in crossover formation than either

315   single mutant (Russo et al., 2023). These data indicate that PCH-2's effect on promoting the removal

316   of GFP::MSH-5 in both the transition zone and mid to late pachytene is through its regulation of HIM-

317   3.

318

319   *pch-2* single mutants exhibit a loss of crossover assurance (Deshong et al., 2014). However, our

320   quantification of GFP::MSH-5 in *pch-2* single mutants in mid to late pachytene showed an increased

321   number of crossover-eligible intermediates (Figure 4A, 4B), suggesting a complex relationship

322   between having too many crossover-eligible intermediates and crossover assurance in *C. elegans*. To

323   address this inconsistency, we generated strains with both OLLAS::COSA-1 (Janisiw et al., 2018) and

324   GFP::MSH-5 in wildtype animals and *pch-2* mutants. Consistent with our and others' previous findings,

325   we observe a gradual reduction in the average number of GFP::MSH-5 foci per row of nuclei in control

326   animals as these nuclei approach the end of pachytene, ultimately converging with the average

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327   number of OLLAS::COSA-1 foci per row (Figure 4E, 4F) (Janisiw et al., 2018; Woglar & Villeneuve,

328   2018). In addition, we consistently observed co-localization of GFP::MSH-5 and OLLAS::COSA-1 foci

329   in control animals, with a few MSH-5 foci persisting without COSA-1 (Figure 4E, 4F).

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331   By contrast, we detected higher average numbers of GFP::MSH-5 foci per row across all late

332   pachytene nuclei in *pch-2* mutants and this average never converged upon the average number of

333   OLLAS-1::COSA-1 foci per row (Figure 4E, 4F). To test whether there was a correlation between the

334   number of GFP::MSH-5 foci and the loss of crossover assurance in *pch-2* mutants, we determined the

335   average number of GFP::MSH-5 foci in meiotic nuclei with 6 OLLAS::COSA-1 foci and those with less

336   than 6 OLLAS::COSA-1 foci in both control and *pch-2* mutant animals (Figure 4E, 4G, Supplemental

337   Figure 2). Indeed, we observed that *pch-2* mutant nuclei with less than 6 OLLAS::COSA-1 foci had

338   significantly higher numbers of GFP::MSH-5 foci, compared to both control and *pch-2* mutant nuclei

339   with 6 OLLAS::COSA-1 foci (Figure 4E, 4G), suggesting that the inability to reduce the number of

340   crossover-eligible intermediates in *pch-2* mutants, counterintuitively, contributes to the observed loss

341   of crossover assurance.

342

343   **PCH-2 is removed from the synaptonemal complex when crossovers are designated**

344

345   We have previously shown that PCH-2 localization to the SC is extended when there are partial

346   defects in synapsis or changes in karyotype, producing an increase in crossovers and a loss of

347   crossover interference (Deshong et al. 2014; Patel et al., 2023). These data led us to hypothesize that

348   PCH-2's presence on chromosomes promotes crossover formation. However, our quantification of

349   GFP::MSH-5 indicates that PCH-2 is required to limit the number of crossover-eligible intermediates,

350   in direct contrast to our proposed hypothesis (Figure 4). Therefore, we decided to revisit what role

351   PCH-2 localization to the SC might play in regulating crossover formation. To this end, we localized

352   PCH-2 in *dsb-2* mutants, in which DSB formation is substantially reduced and fewer crossovers are

353   formed in *C. elegans* (Rosu et al., 2013). We performed this experiment because, in budding yeast,

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354   similar mutants that reduce DSB formation rely on Pch2 to successfully complete meiosis (Joshi et al.,

355   2009; Zanders & Alani, 2009).

356

357   Because the recombination defect in *dsb-2* mutants worsens with age (Rosu et al., 2013), we looked

358   at PCH-2 localization in both young (24 hours post L4 larval stage) and older animals (48 hours post

359   L4). We observed a unique and striking localization pattern in *dsb-2* mutants that we had not observed

360   before. When stained for PCH-2 and GFP::COSA-1, nuclei retain PCH-2 onto chromosomes far into

361   late pachytene, past the normal region when PCH-2 typically is removed from chromosomes (Figure

362   5A, 5B). However, unlike what we have observed in other mutants that have defects in synapsis or

363   changes in karyotype, this retention of PCH-2 is not uniform among all late pachytene nuclei in *dsb-2*

364   mutants. Instead, most nuclei lose PCH-2 localization in late pachytene while some retain it. We

365   tested whether there was a relationship between the retention of PCH-2 and the number of

366   GFP::COSA-1 foci and found that 96% of nuclei that lose PCH-2 have at least 1 GFP::COSA-1 focus

367   (Figure 5A, 5B). In older animals (see 48 hours post-L4), this pattern was even more clear: 77% of

368   nuclei without PCH-2 had 1 or more GFP::COSA-1 focus and 93% of meiotic nuclei without a

369   GFP::COSA-1 focus retained PCH-2.

370

371   These data indicate that PCH-2's removal from the SC is in response to, coincident with or facilitates

372   crossover designation. To distinguish between these possibilities, we analyzed GFP::COSA-1 foci in

373   *dsb-2::AID* (Zhang et al., 2018) and *dsb-2::AID;pch-2* worms, reasoning that if PCH-2's removal is in

374   response to or coincident with designation, we should not detect any differences in the number of

375   GFP::COSA-1 foci in *dsb-2::AID* and *dsb-2::AID;pch-2* worms. We performed these experiments with

376   the auxin-inducible degradation system because of variability in the *dsb-2* mutant background that

377   affected reproducibility of our experiments.

378

15

379 Upon auxin treatment of *dsb-2::AID* worms, we observed a statistically significant (p value < 0.0001,

380 Mann-Whitney U test) decrease in the number of GFP::COSA-1 foci, in comparison with ethanol-

381 treated worms, verifying that we can reliably knock down DSB-2 with the AID system (Figure 5D).

382 When we performed the same experiment in *dsb-2::AID;pch-2* worms, the average number of

383 GFP::COSA-1 foci further decreased, indicating that fewer crossovers are designated when DSBs are

384 reduced and PCH-2 is absent (Figure 5C, 5D). These data suggest that PCH-2's presence on the SC

385 prevents crossover designation. Given the correlation between elevated GFP::MSH-5 foci and the loss

386 of crossover assurance we observe in *pch-2* mutants, we propose that PCH-2 is retained on meiotic

387 chromosomes to ensure that extra crossover-eligible intermediates are removed and crossover

388 designation is delayed until crossover assurance can be guaranteed in *C. elegans*.

389

390 **PCH-2 and high CHK-2 activity control the fate of early double strand breaks**

391

392 In *C. elegans*, meiotic cell cycle entry and progression depends on the activity of CHK-2, the meiosis-

393 specific ortholog of the DNA-damage kinase Chk2/CHEK2 (Baudrimont et al., 2022; Castellano-Pozo

394 et al., 2020; Kim et al., 2015; MacQueen & Villeneuve, 2001; Zhang et al., 2023). Meiotic nuclei in

395 leptotene/zygotene are characterized by high CHK-2 activity, which drops to intermediate activity in

396 mid-pachytene (Kim et al., 2015; Zhang et al., 2023). In late pachytene, CHK-2 activity is inactivated

397 by the recruitment of polo-like kinase, PLK-2, to the SC, which enables crossover designation (Zhang

398 et al., 2023). Thus, CHK-2 activity also has consequences for the progression of meiotic

399 recombination and DNA repair.

400

401 Given that early DSBs are prevented from becoming crossovers by PCH-2 in early meiotic prophase,

402 we wanted to test if high CHK-2 activity in leptotene/zygotene also contributed to the fate of these

403 early DSBs. To evaluate this possibility, we used *syp-1*[T452A] mutants (Sato-Carlton et al., 2018). The

404 SC component, SYP-1, is phosphorylated by CDK-1 on T452, producing a Polo box binding motif that

405 recruits PLK-2 to the SC, contributing to the inactivation of CHK-2 when chromosomes are synapsed

16

406   (Brandt et al., 2020; Zhang et al., 2023). Therefore, in syp-1^{T452A} mutants, CHK-2 activity remains high

407   throughout most of meiotic prophase in the $C.\ elegans$ germline (Figure 6A).

408

409   We quantified the total number of GFP::COSA-1 foci in late pachytene nuclei in syp-1^{T452A} mutants

410   and observed a significant decrease in the average number of GFP::COSA-1 foci when compared to

411   control animals (Figures 6B and 6C), consistent with previous findings that syp-1^{T452A} mutants delay

412   designation and have fewer crossovers (Zhang et al., 2023). These data are also consistent with the

413   possibility that some DSBs fail to become crossovers when CHK-2 activity remains high. If this

414   hypothesis is correct, we predict that the combination of high CHK-2 activity and loss of pch-2 should

415   produce more crossovers. We generated pch-$2;syp$-1^{T452A} double mutants to test this hypothesis.

416   When we quantified GFP::COSA-1 in pch-$2;syp$-1^{T452A} double mutants, we detected a significant

417   increase in the average number of GFP::COSA-1 foci compared to syp-1^{T452A} single mutants (Figure

418   6C), in strong support of our hypothesis.

419

420   To further verify that the pch-2 mutation suppresses the crossover defect in syp-1^{T452A} mutants, we

421   also monitored bivalent formation in diakinesis nuclei. syp-1^{T452A} single mutants exhibit an average of

422   7.12 DAPI staining bodies in diakinesis, higher than both control animals and pch-2 single mutants

423   (Figure 6D). By contrast, pch-$2;syp$-1^{T452A} mutants had an average of 6.05 DAPI staining bodies

424   (Figure 6D, 6E), directly supporting our COSA-1 analysis and indicating that both PCH-2 function and

425   high CHK-2 activity collaborate to control the fate of DSBs and prevent some of them from becoming

426   crossovers in early meiotic prophase.

427

428   **Discussion**

429

430   We have shown that PCH-2 antagonizes crossover formation throughout meiotic prophase and that

431   this regulation occurs through one of the three essential meiotic HORMADs in $C.\ elegans$, HIM-3

432   (Figure 4). We propose that PCH-2 remodels HIM-3 on meiotic chromosomes to destabilize

433   crossover-eligible intermediates, thus limiting which DSBs will become crossovers (Figure 7).

434   However, this antagonism has different consequences depending on when during meiotic prophase it

435   occurs, underscoring the importance of temporal regulation of these events. During

436   leptotene/zygotene, when CHK-2 activity is high (Kim et al., 2015; Zhang et al., 2023) and PCH-2 is

437   present as foci on chromosomes (Deshong et al., 2014), PCH-2 prevents crossover formation at some

438   sites of initial DSB formation and early homolog interactions (Figure 7B). In this way, PCH-2 promotes

439   a wider distribution of crossovers across the genome (Figure 1). In pachytene, when CHK-2 activity

440   has decreased (Kim et al., 2015; Zhang et al., 2023) and PCH-2 is localized to the SC (Deshong et al.,

441   2014), PCH-2 winnows crossover-eligible intermediates, ensuring their reinforcement, designation and

442   crossover assurance (Figure 7B). When there are defects in recombination, such as partial synapsis

443   (Deshong et al., 2014), changes in karyotype (Patel et al., 2023) or too few DSBs (Figure 5), PCH-2

444   persists on the SC to prevent designation, guarantee crossover assurance and some degree of

445   homeostasis, independent of an additional feedback mechanism that increases DSB formation (Patel

446   et al., 2023). That persistence of PCH-2 also disrupts crossover interference in two of these situations

447   (Deshong et al., 2014; Patel et al., 2023) strongly suggests that crossover interference is

448   mechanistically linked to assurance and homeostasis.

449

450   *him-3^{R93Y};pch-2* double mutants have stronger crossover assurance than either single mutant but less

451   than wildtype animals (Russo et al., 2023), a phenotype which can now be explained by the behavior

452   of GFP::MSH-5 foci in these double mutants (Figure 4C, 4D). We have shown that HIM-3^{R93Y} mutant

453   protein can adopt the closed conformation and loads on meiotic chromosomes similar to wildtype HIM-

454   3 (Russo et al. 2023). In vitro analysis shows that HIM-3^{R93Y} binds its closure motif with reduced

455   affinity, likely affecting its ability to adopt the closed conformation in vivo (Figure 7A and Russo et al.

456   2023). The proposed role of PCH-2 in meiosis is to remodel meiotic HORMADs from the closed

457   conformation to the extended one (Figure 7A), regulating their association with chromosomes (Bhalla,

458   2023). However, since meiotic HORMADs are not visibly depleted from chromosomes during meiotic

18

459   progression in *C. elegans* (Couteau et al., 2004; Couteau & Zetka, 2005; Goodyer et al., 2008;

460   Martinez-Perez & Villeneuve, 2005), this genetic interaction supports a role for PCH-2 in temporarily

461   reducing the occupancy of meiotic HORMADs on meiotic chromosomes, destabilizing interactions with

462   partner proteins that modulate the progression and fidelity of meiotic recombination (Russo et al.,

463   2023). Thus, PCH-2's remodeling of HIM-3 would disrupt protein-protein interactions that underlie

464   crossover-eligible intermediates, destabilizing them and reducing their number on chromosomes,

465   contributing to crossover control (Figure 7B). Since meiotic HORMADs are essential for meiotic

466   chromosome axis structure and function, our data therefore support a role for the meiotic axis in

467   crossover control, as suggested by previous reports (Chu et al., 2024; Girard et al., 2023; Lambing et

468   al., 2020; Nabeshima et al., 2004). However, the disassembly of crossover-eligible intermediates

469   would also release pro-crossover factors present at these sites to concentrate at other, more stable

470   sites on the SC, contributing to reinforcement and designation during synapsis (Girard et al., 2023;

471   Yokoo et al., 2012). Moreover, these results raise the possibility that this behavior of PCH-2 and/or

472   meiotic HORMADs might be regulated by post-translational modifications associated with crossover

473   control, such as ubiquitination, SUMOylation and phosphorylation (Gray & Cohen, 2016).

474

475   Given that mutation of PCH-2 produces changes in the number and distribution of crossovers across

476   multiple model systems, we argue that controlling crossover distribution and number is the conserved

477   role of PCH-2 in meiosis (Bhalla, 2023). We have previously proposed that the conserved role of PCH-

478   2 is to coordinate meiotic recombination with synapsis (Bhalla, 2023). With these results, we further

479   refine this model. Early in meiotic prophase, PCH-2 remodels meiotic HORMADs to prevent some

480   DSBs from becoming crossover-eligible intermediates, widening the recombination landscape beyond

481   early homolog interactions and/or sites that tend to be more favorable for DSB formation, also known

482   as 'hot spots." For example, this may explain the localization of TRIP13, the PCH-2 ortholog in mice,

483   to telomeres (Chotiner et al., 2024), sites that experience early homolog interactions due to the

484   organization of meiotic chromosomes in the bouquet formation (Scherthan et al., 1996). This

485   antagonism may also expand the regions of the genome that initiate synapsis in organisms that use

19

486   DSBs to accomplish this event. This possibility is supported by the observation that loss of TRIP13 in

487   mammals produce meiotic chromosomes that exhibit partial asynapsis (Roig et al., 2010), particularly

488   near regions that may act as barriers to SC polymerization (Brown et al., 2005; Roig et al., 2010).

489   Limiting which DSBs becomes crossover-eligible intermediates in early meiotic prophase also ensures

490   that meiotic recombination overlaps with synapsis, either completely, as in *C. elegans* (Yokoo et al.,

491   2012) or partially, as in budding yeast, plants and mice (Capilla-Perez et al., 2021; Cole et al., 2012;

492   Joshi et al., 2015; Morgan et al., 2021). Once synapsis is complete, PCH-2 continues to remodel

493   meiotic HORMADs on chromosomes to control the gradual implementation of crossover number and

494   distribution, reinforcing the important role that synapsis plays in mediating crossover control (Durand

495   et al., 2022; Libuda et al., 2013).

496

497   Unexpectedly, the inability to reduce the number of crossover-eligible intermediates in *pch-2* mutants,

498   as visualized by GFP::MSH-5 foci, does not produce extra crossovers but a loss of crossover-

499   assurance in *C. elegans* (Figure 4), a somewhat counterintuitive result. One interpretation of these

500   data is that crossover-eligible intermediates may be more numerous but absent from some

501   chromosomes in *pch-2* mutants, explaining the loss of crossover assurance. Since the absence of

502   crossover intermediates in *C. elegans* is accompanied by premature desynapsis of individual

503   chromosomes (Machovina et al., 2016; Pattabiraman et al., 2017) and chromosomes in *pch-2* mutants

504   delay desynapsis (Deshong et al., 2014), we do not favor this interpretation. Instead, we propose that

505   having too many crossover-eligible intermediates can be as deleterious to crossover assurance as

506   having too few (Figure 7B). This possibility is further supported by the loss of crossover assurance we

507   detect in irradiated wildtype worms, which is exacerbated in *pch-2* mutants (Figure 2).

508

509   This phenomenon, where crossover-eligible intermediates need to be winnowed to some threshold

510   number to ensure crossover assurance, may also explain the loss of crossover assurance also

511   observed in *Trip13* deficient mice (Roig et al., 2010) and on small chromosomes in budding yeast

20

512 (Chakraborty et al., 2017). Alternatively, the counterintuitive relationship between the number of

513 crossover-eligible precursors and crossover assurance in *pch-2* mutants we observe might reflect an

514 additional layer of regulation during crossover formation specific to *C. elegans*. Since *C. elegans*

515 chromosomes are holocentric, crossovers play an additional role organizing chromosomes for the

516 ordered release of sister chromatid cohesion during meiosis I (Martinez-Perez et al., 2008; Nabeshima

517 et al., 2005) and extra crossovers can be deleterious to accurate chromosome segregation (Hollis et

518 al., 2020). By contrast, in *Arabidopsis*, a system that appears to be able to tolerate an extraordinarily

519 high number of crossovers with little to no effect on chromosome segregation (Durand et al., 2022),

520 PCH2's inability to localize to the SC produces an increase in crossover formation (Figure 7), as

521 visualized by both MLH1 foci and the formation of chiasmata (Yang et al., 2022). Once again, an

522 overarching theme that becomes apparent in our model is that PCH-2 may play a common role in

523 different systems, with dramatic variations in phenotypic consequences given species-specific

524 requirements and constraints.

525

526 We were not surprised to see high numbers of double crossovers on almost every chromosome in our

527 genetic analysis of recombination in wildtype worms, given our previous analysis (Deshong et al.,

528 2014). However, we were surprised to see that the majority of them were found near Pairing Centers

529 and sites of synapsis initiation, suggesting a relationship between early homolog interactions and the

530 formation of double crossovers. When we revisited our previous data, we observed similar patterns. In

531 addition, we do not detect these double crossovers cytologically in *C. elegans*, even when using the

532 OLLAS::COSA-1 reporter, which has been reported to identify double crossovers in spermatogenesis

533 not visualized by GFP::COSA-1 (Cahoon et al., 2023). Crossovers that are cytologically marked by

534 COSA-1 are known as Class I crossovers, which depend on pro-crossover factors such as MSH-5 and

535 ZHP-3, and exhibit crossover control (Gray & Cohen, 2016). These data raise the intriguing possibility

536 that these double crossovers are the product of the alternate, Class II, pathway of crossover

537 formation, which relies on a different suite of proteins, does not respond to crossover control and

538   contributes to varying degrees in different model systems (Gray & Cohen, 2016; Youds et al., 2010).

539   Thus, important corollaries of our model may be that PCH-2 specifically coordinates recombination

540   with synapsis to promote Class I crossovers, limit Class II crossovers and that Class II crossovers are

541   more likely to form early in meiosis, prior to synapsis. Therefore, variations in the contribution of the

542   Class II crossover pathways to crossover recombination and the degree of cross-talk between Class I

543   and Class II pathways among model systems might reflect the degree to which crossover formation

544   overlaps with synapsis (Bhalla, 2023; Gray & Cohen, 2016; Yokoo et al., 2012). Furthermore, these

545   corollaries are entirely consistent with PCH-2's absence from the genome of fission yeast (Wu &

546   Burgess, 2007), a system in which chromosomes do not undergo synapsis, crossovers do not exhibit

547   interference and all crossovers are dependent on the Class II pathway (Hollingsworth & Brill, 2004).

548

549   Our results also have some additional important implications about the regulation of DSB formation

550   across the genome in *C. elegans.* We explain the shift in the recombination landscape away from the

551   central regions of chromosomes and toward PC ends in *pch-2* mutants (Figure 1) as a result of early

552   DSBs becoming crossovers at the expense of later DSBs, suggesting that when DSBs happen in

553   meiotic prophase affects where they happen in the genome. Specifically, we propose that

554   chromosome arms, which are gene poor, receive DSBs early during (or even throughout) DSB

555   formation and the center of chromosomes, which are gene rich, receive DSBs later. A similar

556   regulation of the timing of DSB formation has been demonstrated in budding yeast, where the DSB

557   landscape across the whole genome expands when time in prophase is increased (López Ruiz et al.,

558   2024) and small, highly recombinogenic, chromosomes, get more DSBs later in meiotic prophase

559   (Murakami et al., 2020; Subramanian et al., 2019). However, this temporal regulation has not been

560   reported previously in *C. elegans* and suggests that this phenomenon is more widely conserved. This

561   expansion of the DSB landscape in *C. elegans* to include the center regions of chromosomes later in

562   meiosis may be a deliberate attempt for recombination to create new haplotypes for evolution to act

563   on, despite the relative paucity of DSBs and the observation that they can result in chromosome

564   missegregation (Altendorfer et al., 2020).

565

566 Finally, our work raises some important questions about the functional role(s) of DSBs in meiosis,

567 aside from their contributions to crossover formation. Hicks and colleagues were the first to report that

568 early DSBs do not contribute to crossover formation in *C. elegans* (Hicks et al., 2022). Here we show

569 that these early DSBs are prevented from becoming crossovers by both PCH-2 activity and cell cycle

570 stage, specifically in leptotene/zygotene, when homologs are initiating pairing and synapsis. In

571 budding yeast, similar, early-occurring DSBs have been characterized as "scout DSBs" because of

572 their preference for repair from sister chromatids, versus homologous chromosomes, and their

573 proposed role in contributing to homolog pairing (Borde & de Massy, 2015; Joshi et al., 2015). The

574 homolog bias that these "scout DSBs" do display seems dependent on budding yeast *PCH2* (Joshi et

575 al., 2015) but interpreting this experiment is complicated by the fact that Pch2 is also required to make

576 the budding yeast meiotic HORMAD, Hop1, available for its loading onto meiotic chromosomes

577 (Herruzo et al., 2021).

578

579 In contrast to budding yeast, *C. elegans* does not rely on DSBs to promote homolog pairing and

580 initiate synapsis (Dernburg et al., 1998); in worms, *cis*-acting sites called Pairing Centers are essential

581 for homolog pairing and synapsis (MacQueen et al., 2005). There is certainly some support in the

582 literature for DSBs playing a role in supporting pairing and synapsis in *C. elegans* (Guo et al., 2022;

583 Mlynarczyk-Evans et al., 2013; Roelens et al., 2015). However, if these early DSBs were contributing

584 to pairing and synapsis, we would expect to see a genetic interaction between *htp-3^{H96Y}* and *pch-2*

585 mutations in the installation of GFP::MSH-5 in the transition zone; we previously reported that *htp-*

586 *3^{H96Y}* suppresses the acceleration of pairing and synapsis of *pch-2* mutants, particularly when Pairing

587 Center function is compromised (Russo et al., 2023). Instead, we favor the possibility that these early

588 DSBs are generated to amplify the signaling of DNA damage kinases to prime them for their role in

589 recombination, a role that has also been proposed for "scout" DSBs (Joshi et al., 2015). That ATM-1, a

590 conserved DNA damage kinase that is downstream of CHK-2 in *C. elegans*, relies on DSBs for full

591 activity, supports this proposed, conserved role (Yu et al., 2023).

23

592

593   Our work provides an important framework to finally understand the role of PCH-2 in controlling the

594   number and distribution of crossovers, a role that we argue is its conserved role. While specific details

595   may vary across systems, we propose that PCH-2 remodels meiotic HORMADs throughout meiotic

596   prophase to destabilize crossover-eligible precursors, coordinating meiotic recombination with

597   synapsis, contributing to the progressive implementation of meiotic recombination and guaranteeing

598   crossover assurance, interference and homeostasis.

599

600   **Methods and Materials**

601

602   ***C.elegans* Genetics and Genome Engineering**

603   The *C. elegans* Bristol N2 was used as the wild-type strain. All strains were maintained at 20ºC under

604   standard conditions unless stated. Mutant combinations were generated by crossing. The following

605   mutants and rearrangements were used:

606

607   *LGII: pch-2(tm1458), meIs8 ([pie-1p::GFP::cosa-1 + unc-119(+)], dsb-2(me96), dsb-2(ie58[dsb-*

608   *2::AID::3xFLAG])*

609

610   *LGIII: cosa-1(ddr12[OLLAS::cosa-1]); htp-3(vc75)*

611

612   *LGIV: him-3(blt9), spo-11(ie59[spo-11::AID::3xFLAG]), msh-5[ddr22(GFP::msh-5)], ieSi38 [sun-*

613   *1p::TIR1::mRuby::sun-1 3'UTR + Cbr-unc-119(+)]*

614

615   *LGV: syp-1(icm85[T452A]), nT1[qIs51], bcIs39 (Plin-15::ced-1::GFP)*

616

617   **Genetic analysis of Recombination**

618   The wildtype Hawaiian CB4856 strain (HI) and the Bristol N2 strain were used to assay recombination

619   between single nucleotide polymorphisms (SNPs) on Chromosomes I, III, IV and X (Wicks et al.,

620   2001; Bazan et al., 2011). The SNPs, primers, enzymes used for restriction digests and expected

621   fragment sizes are included in Supplemental Table 1. To measure wild-type recombination, N2 males

622   containing *bcls39* were crossed to Hawaiian CB4856 worms. Cross-progeny hermaphrodites were

623   identified by the presence of *bcls39* and contained one N2 and one CB4856 chromosome.

624

625   These were assayed for recombination by crossing with CB4856 males containing *myo-2::mCherry*.

626   Cross-progeny hermaphrodites from the resulting cross were isolated as L4s, and then cultured

627   individually in 96- well plates in liquid S-media complete supplemented with HB101. Four days after

628   initial culturing, starved populations were lysed and used for PCR and restriction digest to detect N2

629   and CB4856 SNP alleles. For recombination in *pch-2* mutants, strains homozygous for the CB4856

630   background of the relevant SNPs were created, then mated with *pch-2; bcls39*. Subsequent steps

631   were performed as in the wild-type worms.

632

633   **Immunostaining**

634   DAPI staining and immunostaining was performed as in (Russo et al. 2023), 20 to 24 hours post L4

635   unless otherwise noted. For analyzing bivalents, the same protocol was implemented with the

636   exception that hermaphrodites were dissected and DAPI stained 48 hours post late L4 stage, unless

637   otherwise noted.

638

639   The following primary antibodies were used at the indicated dilutions: alpaca anti-GFP Booster

640   (ChromoTek, gb2AF488) was used at 1:1000; rat anti-OLLAS (Invitrogen, PIMA516125) was used at

641   1:1000; and rabbit anti PCH-2 (Bhalla 2014) was used at 1:500. The following secondary antibodies

642   were used at the indicated dilutions: anti-rabbit Cy3 (Jackson Labs) was used at 1:500 and anti-rat

643   Cy5 (Jackson Labs) was used at 1:500

644

Irradiation Experiments

646 Control and *pch-2* mutant L4's were aged 12-14 hours before being exposed to 1,000 rad (10 Gy) of

647 X-ray radiation using a Precision MultiRad 160 X-irradiator (Precision X-Ray Inc.). Germlines were

648 then fixed and stained, 8 hours and 24 hours post irradiation.

649

Auxin-induced Degradation Experiments

651 Auxin treatment was performed by transferring young adult worms (aged 12-14 hours post-L4) to

652 bacteria-seeded plates containing auxin or 99% ethanol at specific time points, except for experiments

653 in which L4s were transferred directly to bacteria-seeded plates containing auxin or ethanol (the 36

654 hour and 48 hour time points in Figures 3C and E and the experiments in Figures 5C and D). The

655 natural auxin indole3-acetic acid (IAA) was purchased from Alfa Aesar (#A10556). A 400 mM stock

656 solution in ethanol was prepared and was stored at 4ºC for up to one month. Auxin was diluted to

657 100mM, and 100ul was spread onto NGM plates. Plates were allowed to dry before seeding with fresh

658 OP50 culture. Plates were left at 20ºC for 2-3 days in the dark to allow for bacterial lawn growth.

659

Imaging and Quantification

661 All images were acquired using a DeltaVision Personal DV system (Applied Precision) equipped with a

662 100X N.A. 1.40 oil-immersion objective (Olympus), resulting in an effective XY pixel spacing of 0.064

663 or 0.040 µm. Three-dimensional image stacks were collected at 0.2-µm Z spacing and processed by

664 constrained, iterative deconvolution. Image scaling, analysis and maximum-intensity projections were

665 performed using functions in the softWoRx software package.

666

667 For analysis of GFP::MSH-5 foci, sum projections were generated using ImageJ for each image of the

668 germline. ImageJ plugins Cell Counter, ROI Manager, and Find Maxima were used to identify and

669 quantify GFP::MSH-5 foci by row from transition zone to the end of pachytene. The threshold value

670 was set depending on background conditions to ensure minimal signals were identified. Foci were

26

671   only quantified if they co-localized with DAPI staining. For all genotypes, three germlines per genotype

672   were analyzed and representative germlines are shown.

673

674   **Graphing and Statistical Analysis**

675   Data was analyzed using Python 3.8 and Prism for statistical significance. All datasets were tested for

676   normality using the Shapiro-Wilk test. For Figures 1, 2 and 5B, Fisher's exact test was used to

677   determine significance. For Figures 3, 4, 5D and 6, Mann-Whitney U test was used to determine

678   significance.

679

680   **Author contributions**

681   B.P., M.G., and N.B. designed the experiments. B.P., M.G., A.H., E.L., and V.O. performed the

682   experiments. B.P. and N.B. analyzed the data. B.P. and N.B. wrote the initial draft of the manuscript

683   and B.P., M.G., A.H. and N.B. revised the manuscript. N.B. acquired funding.

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References

Altendorfer, E., Láscarez-Lagunas, L. I., Nadarajan, S., Mathieson, I., & Colaiácovo, M. P. (2020).

Crossover Position Drives Chromosome Remodeling for Accurate Meiotic Chromosome Segregation.

Current Biology, 30(7), 1329-1338.e7. https://doi.org/10.1016/j.cub.2020.01.079

Baudrimont, A., Paouneskou, D., Mohammad, A., Lichtenberger, R., Blundon, J., Kim, Y., Hartl, M.,

Falk, S., Schedl, T., & Jantsch, V. (2022). Release of CHK-2 from PPM-1.D anchorage schedules

meiotic entry. *Science Advances*, 8(7), eabl8861. https://doi.org/10.1126/sciadv.abl8861

Bhalla, N. (2023). PCH-2 and meiotic HORMADs: A module for evolutionary innovation in meiosis? *In*

Current Topics in Developmental Biology (Vol. 151, pp. 317–344). Elsevier.

https://doi.org/10.1016/bs.ctdb.2022.07.001

Borde, V., & deMassy, B. (2015). Meiosis: Early DNA Double-Strand Breaks Pave the Way for Inter-

Homolog Repair. *Developmental Cell*, 32(6), 663–664. https://doi.org/10.1016/j.devcel.2015.03.011

Börner, G. V., Barot, A., & Kleckner, N. (2008). Yeast Pch2 promotes domainal axis organization,

timely recombination progression, and arrest of defective recombinosomes during meiosis.

Proceedings of the National Academy of Sciences, 105(9), 3327–3332.

https://doi.org/10.1073/pnas.0711864105

Brandt, J. N., Hussey, K. A., & Kim, Y. (2020). Spatial and temporal control of targeting Polo-like

kinase during meiotic prophase. *Journal of Cell Biology*, 219(11), e202006094.

https://doi.org/10.1083/jcb.202006094

717 Brown, P. W., Judis, L., Chan, E. R., Schwartz, S., Seftel, A., Thomas, A., & Hassold, T. J. (2005).

718 Meiotic Synapsis Proceeds from a Limited Number of Subtelomeric Sites in the Human Male. *The*

719 *American Journal of Human Genetics*, 77(4), 556–566. https://doi.org/10.1086/468188

720

721 Cahoon CK, Uebel CJ, Villeneuve AM, Libuda DE. Epitope tag-specific differences in the detection of

722 COSA-1 marked crossover sites in *C. elegans* spermatocytes. *MicroPubl Biol.* 2023 Jan

723 6;2023:10.17912/micropub.biology.000724. https://doi.org/10.17912/micropub.biology.000724.

724

725 Capilla-Pérez, L., Durand, S., Hurel, A., Lian, Q., Chambon, A., Taochy, C., Solier, V., Grelon, M., &

726 Mercier, R. (2021). The synaptonemal complex imposes crossover interference and heterochiasmy in

727 Arabidopsis. *Proceedings of the National Academy of Sciences*, 118(12), e2023613118.

728 https://doi.org/10.1073/pnas.2023613118

729

730 Castellano-Pozo, M., Pacheco, S., Sioutas, G., Jaso-Tamame, A. L., Dore, M. H., Karimi, M. M., &

731 Martinez-Perez, E. (2020). Surveillance of cohesin-supported chromosome structure controls meiotic

732 progression. *Nature Communications*, 11(1), 4345. https://doi.org/10.1038/s41467-020-18219-9

733

734 Chakraborty, P., Pankajam, A. V., Lin, G., Dutta, A., Krishnaprasad, G. N., Tekkedil, M. M., Shinohara,

735 A., Steinmetz, L. M., & Nishant, K. T. (2017). Modulating Crossover Frequency and Interference for

736 Obligate Crossovers in Saccharomyces cerevisiae Meiosis. *Genes|Genomes|Genetics*, 7(5), 1511–

737 1524. https://doi.org/10.1534/g3.117.040071

738

739 Chotiner, J. Y., Leu, N. A., Yang, F., Cossu, I. G., Guan, Y., Lin, H., & Wang, P. J. (2024). TRIP13

740 localizes to synapsed chromosomes and functions as a dosage-sensitive regulator of meiosis.

741 https://doi.org/10.7554/eLife.92195.2

742

743

744 Chu, L., Zhuang, J., Geng, M., Zhang, Y., Zhu, J., Zhang, C., Schnittger, A., Yi, B., & Yang, C. (2024).

745 ASYNAPSIS3 has diverse dosage-dependent effects on meiotic crossover formation in Brassica

746 napus. *The Plant Cell*, koae207. https://doi.org/10.1093/plcell/koae207

747

748 Cole, F., Kauppi, L., Lange, J., Roig, I., Wang, R., Keeney, S., & Jasin, M. (2012). Homeostatic control

749 of recombination is implemented progressively in mouse meiosis. *Nature Cell Biology*, 14(4), 424–

750 430. https://doi.org/10.1038/ncb2451

751

752 Couteau, F., Nabeshima, K., Villeneuve, A., & Zetka, M. (2004). A Component of *C. elegans* Meiotic

753 Chromosome Axes at the Interface of Homolog Alignment, Synapsis, Nuclear Reorganization, and

754 Recombination. *Current Biology*, 14(7), 585–592. https://doi.org/10.1016/j.cub.2004.03.033

755

756 Couteau, F., & Zetka, M. (2005). HTP-1 coordinates synaptonemal complex assembly with homolog

757 alignment during meiosis in *C. elegans. Genes & Development*, 19(22), 2744–2756.

758 https://doi.org/10.1101/gad.1348205

759

760 Cuacos, M., Lambing, C., Pachon-Penalba, M., Osman, K., Armstrong, S. J., Henderson, I. R.,

761 Sanchez-Moran, E., Franklin, F. C. H., & Heckmann, S. (2021). Meiotic chromosome axis remodelling

762 is critical for meiotic recombination in *Brassica rapa. Journal of Experimental Botany*, 72(8), 3012–

763 3027. https://doi.org/10.1093/jxb/erab035

764

765 Dernburg, A. F., McDonald, K., Moulder, G., Barstead, R., Dresser, M., & Villeneuve, A. M. (1998).

766 Meiotic Recombination in *C. elegans* Initiates by a Conserved Mechanism and Is Dispensable for

767 Homologous Chromosome Synapsis. *Cell*, 94(3), 387–398. https://doi.org/10.1016/S0092-

768 8674(00)81481-6

769

770   Deshong, A. J., Ye, A. L., Lamelza, P., & Bhalla, N. (2014). A Quality Control Mechanism Coordinates

771   Meiotic Prophase Events to Promote Crossover Assurance. *PLoS Genetics*, 10(4), e1004291.

772   https://doi.org/10.1371/journal.pgen.1004291

773

774   Durand, S., Lian, Q., Jing, J., Ernst, M., Grelon, M., Zwicker, D., & Mercier, R. (2022). Joint control of

775   meiotic crossover patterning by the synaptonemal complex and HEI10 dosage. *Nature*

776   *Communications*, 13(1), 5999. https://doi.org/10.1038/s41467-022-33472-w

777

778   Girard, C., Zwicker, D., & Mercier, R. (2023). The regulation of meiotic crossover distribution: A coarse

779   solution to a century-old mystery? *Biochemical Society Transactions*, 51(3), 1179–1190.

780   https://doi.org/10.1042/BST20221329

781

782   Goodyer, W., Kaitna, S., Couteau, F., Ward, J. D., Boulton, S. J., & Zetka, M. (2008). HTP-3 Links

783   DSB Formation with Homolog Pairing and Crossing Over during *C. elegans* Meiosis. *Developmental*

784   *Cell*, 14(2), 263–274. https://doi.org/10.1016/j.devcel.2007.11.016

785

786   Gray, S., & Cohen, P. E. (2016). Control of Meiotic Crossovers: From Double-Strand Break Formation

787   to Designation. *Annual Review of Genetics*, 50(1), 175–210. https://doi.org/10.1146/annurev-genet-

788   120215-035111

789

790   Gu, Y., Desai, A., & Corbett, K. D. (2022). Evolutionary Dynamics and Molecular Mechanisms of

791   HORMA Domain Protein Signaling. *Annual Review of Biochemistry*, 91(1), 541–569.

792   https://doi.org/10.1146/annurev-biochem-090920-103246

793

794   Guo, H., Stamper, E. L., Sato-Carlton, A., Shimazoe, M. A., Li, X., Zhang, L., Stevens, L., Jacky Tam,

795   K., Dernburg, A. F., & Carlton, P. M. (2022). Phosphoregulation of DSB-1 mediates control of meiotic

796   double-strand break activity. *eLife*, 11, e77956. https://doi.org/10.7554/eLife.77956

797

798   Hassold, T., & Hunt, P. (2001). To err (meiotically) is human: The genesis of human aneuploidy. Nature

799   Reviews *Genetics*, 2(4), 280–291. https://doi.org/10.1038/35066065

800

801   Haversat, J., Woglar, A., Klatt, K., Akerib, C. C., Roberts, V., Chen, S.-Y., Arur, S., Villeneuve, A. M., &

802   Kim, Y. (2022). Robust designation of meiotic crossover sites by CDK-2 through phosphorylation of the

803   MutSγ complex. *Proceedings of the National Academy of Sciences*, 119(21), e2117865119.

804   https://doi.org/10.1073/pnas.2117865119

805

806   Herruzo, E., Lago-Maciel, A., Baztán, S., Santos, B., Carballo, J. A., & San-Segundo, P. A. (2021).

807   Pch2 orchestrates the meiotic recombination checkpoint from the cytoplasm. *PLOS Genetics*, 17(7),

808   e1009560. https://doi.org/10.1371/journal.pgen.1009560

809

810   Hicks, T., Trivedi, S., Eppert, M., Bowman, R., Tian, H., Dafalla, A., Crahan, C., Smolikove, S., & Silva,

811   N. (2022). Continuous double-strand break induction and their differential processing sustain chiasma

812   formation during *Caenorhabditis elegans* meiosis. *Cell Reports*, 40(13), 111403.

813   https://doi.org/10.1016/j.celrep.2022.111403

814

815   Hollingsworth, N. M., & Brill, S. J. (2004). The Mus81 solution to resolution: Generating meiotic

816   crossovers without Holliday junctions. *Genes & Development*, 18(2), 117–125.

817   https://doi.org/10.1101/gad.1165904

818

819   Hollis, J. A., Glover, M. L., Schlientz, A. J., Cahoon, C. K., Bowerman, B., Wignall, S. M., & Libuda, D.

820   E. (2020). Excess crossovers impede faithful meiotic chromosome segregation in *C. elegans*. *PLOS*

821   *Genetics*, 16(9), e1009001. https://doi.org/10.1371/journal.pgen.1009001

822

823 Janisiw, E., Dello Stritto, M. R., Jantsch, V., & Silva, N. (2018). BRCA1-BARD1 associate with the

824 synaptonemal complex and pro-crossover factors and influence RAD-51 dynamics during

825 *Caenorhabditis elegans* meiosis. *PLOS Genetics*, 14(11), e1007653.

826 https://doi.org/10.1371/journal.pgen.1007653

827

828 Jaramillo-Lambert, A., Ellefson, M., Villeneuve, A. M., & Engebrecht, J. (2007). Differential timing of S

829 phases, X chromosome replication, and meiotic prophase in the *C. elegans* germ line. *Developmental*

830 *Biology*, *308*(1), 206–221. https://doi.org/10.1016/j.ydbio.2007.05.019

831

832 Joshi, N., Barot, A., Jamison, C., & Börner, G. V. (2009). Pch2 Links Chromosome Axis Remodeling at

833 Future Crossover Sites and Crossover Distribution during Yeast Meiosis. *PLOS Genetics*, 5(7),

834 e1000557. https://doi.org/10.1371/journal.pgen.1000557

835

836 Joshi, N., Brown, M. S., Bishop, D. K., & Börner, G. V. (2015). Gradual Implementation of the Meiotic

837 Recombination Program via Checkpoint Pathways Controlled by Global DSB Levels. *Molecular Cell*,

838 57(5), 797–811. https://doi.org/10.1016/j.molcel.2014.12.027

839

840 Joyce, E. F., & McKim, K. S. (2009). Drosophila PCH2 Is Required for a Pachytene Checkpoint That

841 Monitors Double-Strand-Break-Independent Events Leading to Meiotic Crossover Formation.

842 *Genetics*, 181(1), 39–51. https://doi.org/10.1534/genetics.108.093112

843

844 Keeney S, Giroux CN, Kleckner N. Meiosis-specific DNA double-strand breaks are catalyzed by

845 Spo11, a member of a widely conserved protein family. *Cell*. 1997 Feb 7;88(3):375-84. doi:

846 10.1016/s0092-8674(00)81876-0. PMID: 9039264.

847

848 Kelly, K. O., Dernburg, A. F., Stanfield, G. M., & Villeneuve, A. M. (2000). *Caenorhabditis elegans* msh-

849 5 Is Required for Both Normal and Radiation-Induced Meiotic Crossing Over but Not for Completion of

850 Meiosis. *Genetics*, 156(2), 617–630. https://doi.org/10.1093/genetics/156.2.617

851

852 Kim, Y., Kostow, N., & Dernburg, A. F. (2015). The Chromosome Axis Mediates Feedback Control of

853 CHK-2 to Ensure Crossover Formation in *C. elegans. Developmental Cell*, 35(2), 247–261.

854 https://doi.org/10.1016/j.devcel.2015.09.021

855

856 Kim, Y., Rosenberg, S. C., Kugel, C. L., Kostow, N., Rog, O., Davydov, V., Su, T. Y., Dernburg, A. F., &

857 Corbett, K. D. (2014). The Chromosome Axis Controls Meiotic Events through a Hierarchical Assembly

858 of HORMA Domain Proteins. *Developmental Cell*, 31(4), 487–502.

859 https://doi.org/10.1016/j.devcel.2014.09.013

860

861 Lambing, C., Kuo, P. C., Tock, A. J., Topp, S. D., & Henderson, I. R. (2020). ASY1 acts as a dosage-

862 dependent antagonist of telomere-led recombination and mediates crossover interference in

863 *Arabidopsis. Proceedings of the National Academy of Sciences*, 117(24), 13647–13658.

864 https://doi.org/10.1073/pnas.1921055117

865

866 Lambing, C., Osman, K., Nuntasoontorn, K., West, A., Higgins, J. D., Copenhaver, G. P., Yang, J.,

867 Armstrong, S. J., Mechtler, K., Roitinger, E., & Franklin, F. C. H. (2015). *Arabidopsis* PCH2 Mediates

868 Meiotic Chromosome Remodeling and Maturation of Crossovers. *PLOS Genetics*, 11(7), e1005372.

869 https://doi.org/10.1371/journal.pgen.1005372

870

871 Libuda, D. E., Uzawa, S., Meyer, B. J., & Villeneuve, A. M. (2013). Meiotic chromosome structures

872 constrain and respond to designation of crossover sites. *Nature*, 502(7473), 703–706.

873 https://doi.org/10.1038/nature12577

874

875 López Ruiz, L. M., Johnson, D., Gittens, W. H., Brown, G. G. B., Allison, R. M., & Neale, M. J. (2024).

876 Meiotic prophase length modulates Tel1-dependent DNA double-strand break interference. PLOS

877 *Genetics*, 20(3), e1011140. https://doi.org/10.1371/journal.pgen.1011140

878

879 Machovina, T. S., Mainpal, R., Daryabeigi, A., McGovern, O., Paouneskou, D., Labella, S., Zetka, M.,

880 Jantsch, V., Yanowitz, J. L. (2016). A Surveillance System Ensures Crossover Formation in

881 *C. elegans. Current Biology*, 26(21), 2873-2884. https://doi.org/10.1016/j.cub.2016.09.007

882

883 MacQueen, A. J., Phillips, C. M., Bhalla, N., Weiser, P., Villeneuve, A. M., & Dernburg, A. F. (2005).

884 Chromosome Sites Play Dual Roles to Establish Homologous Synapsis during Meiosis in *C. elegans*.

885 *Cell*, 123(6), 1037–1050. https://doi.org/10.1016/j.cell.2005.09.034

886

887 MacQueen, A. J., & Villeneuve, A. M. (2001). Nuclear reorganization and homologous chromosome

888 pairing during meiotic prophase require *C. elegans chk-2. Genes & Development*, 15(13), 1674–1687.

889 https://doi.org/10.1101/gad.902601

890

891 Martinez-Perez, E., Schvarzstein, M., Barroso, C., Lightfoot, J., Dernburg, A. F., & Villeneuve, A. M.

892 (2008). Crossovers trigger a remodeling of meiotic chromosome axis composition that is linked to two-

893 step loss of sister chromatid cohesion. *Genes & Development*, 22(20), 2886–2901.

894 https://doi.org/10.1101/gad.1694108

895

896 Martinez-Perez, E., & Villeneuve, A. M. (2005). HTP-1-dependent constraints coordinate homolog

897 pairing and synapsis and promote chiasma formation during *C. elegans* meiosis. *Genes &*

898 *Development*, 19(22), 2727–2743. https://doi.org/10.1101/gad.1338505

899

900   Mlynarczyk-Evans, S., Roelens, B., & Villeneuve, A. M. (2013). Evidence That Masking of Synapsis

901   Imperfections Counterbalances Quality Control to Promote Efficient Meiosis. *PLoS Genetics*, 9(12),

902   e1003963. https://doi.org/10.1371/journal.pgen.1003963

903

904   Morgan, C., White, M. A., Franklin, F. C. H., Zickler, D., Kleckner, N., & Bomblies, K. (2021). Evolution

905   of crossover interference enables stable autopolyploidy by ensuring pairwise partner connections in

906   *Arabidopsis arenosa*. *Current Biology*, 31(21), 4713-4726.e4.

907   https://doi.org/10.1016/j.cub.2021.08.028

908

909   Murakami, H., Lam, I., Huang, P.-C., Song, J., Van Overbeek, M., & Keeney, S. (2020). Multilayered

910   mechanisms ensure that short chromosomes recombine in meiosis. *Nature*, 582(7810), 124–128.

911   https://doi.org/10.1038/s41586-020-2248-2

912

913   Nabeshima, K., Villeneuve, A. M., & Colaiácovo, M. P. (2005). Crossing over is coupled to late meiotic

914   prophase bivalent differentiation through asymmetric disassembly of the SC. *The Journal of Cell*

915   *Biology*, 168(5), 683–689. https://doi.org/10.1083/jcb.200410144

916

917   Nabeshima, K., Villeneuve, A. M., & Hillers, K. J. (2004). Chromosome-Wide Regulation of Meiotic

918   Crossover Formation in *Caenorhabditis elegans* Requires Properly Assembled Chromosome Axes.

919   *Genetics*, 168(3), 1275–1292. https://doi.org/10.1534/genetics.104.030700

920

921   Nadarajan, S., Altendorfer, E., Saito, T. T., Martinez-Garcia, M., & Colaiácovo, M. P. (2021). HIM-17

922   regulates the position of recombination events and GSP-1/2 localization to establish short arm identity

923   on bivalents in meiosis. *Proceedings of the National Academy of Sciences*, 118(17), e2016363118.

924   https://doi.org/10.1073/pnas.2016363118

925

926   Patel, B; Grobler, M; Bhalla, N (2023). Chromosomal fusions, but not chromosomal inversions,

927   activate a PCH-2 dependent checkpoint that promotes crossover formation in *C. elegans*.

928   *microPublication Biology*.

929

930   Pattabiraman, D., Roelens, B., Woglar, A., Villeneuve A. M. (2017) Meiotic recombination modulates

931   the structure and dynamics of the synaptonemal complex during *C. elegans* meiosis. *PLOS Genetics*,

932   13(3), e1006670. https://doi.org/10.1371/journal.pgen.1006670

933

934   Roelens, B., Schvarzstein, M., & Villeneuve, A. M. (2015). Manipulation of Karyotype in

935   *Caenorhabditis elegans* Reveals Multiple Inputs Driving Pairwise Chromosome Synapsis During

936   Meiosis. *Genetics*, 201(4), 1363–1379. https://doi.org/10.1534/genetics.115.182279

937

938   Roig, I., Dowdle, J. A., Toth, A., de Rooij, D. G., Jasin, M., & Keeney, S. (2010). Mouse TRIP13/PCH2

939   Is Required for Recombination and Normal Higher-Order Chromosome Structure during Meiosis.

940   *PLOS Genetics,* 6(8), e1001062. https://doi.org/10.1371/journal.pgen.1001062

941

942   Rosu, S., Zawadzki, K. A., Stamper, E. L., Libuda, D. E., Reese, A. L., Dernburg, A. F., & Villeneuve, A.

943   M. (2013). The *C. elegans* DSB-2 Protein Reveals a Regulatory Network that Controls Competence

944   for Meiotic DSB Formation and Promotes Crossover Assurance. *PLOS Genetics*, 9(8), e1003674.

945   https://doi.org/10.1371/journal.pgen.1003674

946

947   Russo, A. E., Giacopazzi, S., Deshong, A., Menon, M., Ortiz, V., Ego, K. M., Corbett, K. D., & Bhalla,

948   N. (2023). The conserved AAA ATPase PCH-2 distributes its regulation of meiotic prophase events

949   through multiple meiotic HORMADs in *C. elegans*. *PLOS Genetics*, 19(4), e1010708.

950   https://doi.org/10.1371/journal.pgen.1010708

951

952   Sato-Carlton, A., Nakamura-Tabuchi, C., Chartrand, S. K., Uchino, T., & Carlton, P. M. (2018).

953   Phosphorylation of the synaptonemal complex protein SYP-1 promotes meiotic chromosome

954   segregation. *Journal of Cell Biology*, 217(2), 555–570. https://doi.org/10.1083/jcb.201707161

955

956   Scherthan, H., Weich, S., Schwegler, H., Heyting, C., Härle, M., & Cremer, T. (1996). Centromere and

957   telomere movements during early meiotic prophase of mouse and man are associated with the onset

958   of chromosome pairing. *The Journal of Cell Biology*, 134(5), 1109–1125.

959   https://doi.org/10.1083/jcb.134.5.1109

960

961   Snowden, T., Acharya, S., Butz, C., Berardini, M., & Fishel, R. (2004). hMSH4-hMSH5 Recognizes

962   Holliday Junctions and Forms a Meiosis-Specific Sliding Clamp that Embraces Homologous

963   Chromosomes. *Molecular Cell*, 15(3), 437–451. https://doi.org/10.1016/j.molcel.2004.06.040

964

965   Subramanian, V. V., Zhu, X., Markowitz, T. E., Vale-Silva, L. A., San-Segundo, P. A., Hollingsworth, N.

966   M., Keeney, S., & Hochwagen, A. (2019). Persistent DNA-break potential near telomeres increases

967   initiation of meiotic recombination on short chromosomes. *Nature Communications*, 10(1), 970.

968   https://doi.org/10.1038/s41467-019-08875-x

969

970   Wicks, S. R., Yeh, R. T., Gish, W. R., Waterston, R. H., & Plasterk, R. H. A. (2001). Rapid gene

971   mapping in *Caenorhabditis elegans* using a high density polymorphism map. *Nature Genetics*, 28(2),

972   160–164. https://doi.org/10.1038/88878

973

974   Woglar, A., & Villeneuve, A. M. (2018). Dynamic Architecture of DNA Repair Complexes and the

975   Synaptonemal Complex at Sites of Meiotic Recombination. *Cell*, 173(7), 1678-1691.e16.

976   https://doi.org/10.1016/j.cell.2018.03.066

977   Wojtasz, L., Daniel, K., Roig, I., Bolcun-Filas, E., Xu, H., Boonsanay, V., Eckmann, C. R., Cooke, H. J.,

978   Jasin, M., Keeney, S., McKay, M. J., & Toth, A. (2009). Mouse HORMAD1 and HORMAD2, Two

979   Conserved Meiotic Chromosomal Proteins, Are Depleted from Synapsed Chromosome Axes with the

980   Help of TRIP13 AAA-ATPase. *PLOS Genetics*, 5(10), e1000702.

981   https://doi.org/10.1371/journal.pgen.1000702

982

983   Wu, H. Y., & Burgess, S. M. (2006). Two distinct surveillance mechanisms monitor meiotic

984   chromosome metabolism in budding yeast. *Current biology*: CB, 16(24), 2473–2479.

985   https://doi.org/10.1016/j.cub.2006.10.069

986

987   Yang, C., Hu, B., Portheine, S. M., Chuenban, P., & Schnittger, A. (2020). State changes of the

988   HORMA protein ASY1 are mediated by an interplay between its closure motif and PCH2. *Nucleic*

989   *Acids Research*, 48(20), 11521-11535. https://doi.org/10.1093/nar/gkaa527

990

991   Yang, C., Sofroni, K., Hamamura, Y., Hu, B., Elbasi, H. T., Balboni, M., Chu, L., Stang, D., Heese, M.,

992   & Schnittger, A. (2022). ZYP1-mediated recruitment of PCH2 to the synaptonemal complex remodels

993   the chromosome axis leading to crossover restriction. *Nucleic Acids Research*, 50(22), 12924–12937.

994   https://doi.org/10.1093/nar/gkac1160

995

996   Yokoo, R., Zawadzki, K. A., Nabeshima, K., Drake, M., Arur, S., & Villeneuve, A. M. (2012). COSA-1

997   Reveals Robust Homeostasis and Separable Licensing and Reinforcement Steps Governing Meiotic

998   Crossovers. *Cell*, 149(1), 75–87. https://doi.org/10.1016/j.cell.2012.01.052

999

1000   Youds, J. L., Mets, D. G., McIlwraith, M. J., Martin, J. S., Ward, J. D., ONeil, N. J., Rose, A. M., West,

1001   S. C., Meyer, B. J., & Boulton, S. J. (2010). RTEL-1 Enforces Meiotic Crossover Interference and

1002   Homeostasis. *Science*, 327(5970), 1254–1258. https://doi.org/10.1126/science.1183112

1003

1004   Yu, Z., Kim, H. J., & Dernburg, A. F. (2023). ATM signaling modulates cohesin behavior in meiotic

1005   prophase and proliferating cells. *Nature Structural & Molecular Biology*, 30(4), 436–450.

1006   https://doi.org/10.1038/s41594-023-00929-5

1007

1008   Yu, Z., Kim, Y., & Dernburg, A. F. (2016). Meiotic recombination and the crossover assurance

1009   checkpoint in *Caenorhabditis elegans. Seminars in Cell & Developmental Biology*, 54, 106–116.

1010   https://doi.org/10.1016/j.semcdb.2016.03.014

1011

1012   Zanders, S., & Alani, E. (2009). The *pch2Δ* Mutation in Baker's Yeast Alters Meiotic Crossover Levels

1013   and Confers a Defect in Crossover Interference. *PLOS Genetics*, 5(7), e1000571.

1014   https://doi.org/10.1371/journal.pgen.1000571

1015

1016   Zhang, L., Köhler, S., Rillo-Bohn, R., & Dernburg, A. F. (2018). A compartmentalized signaling network

1017   mediates crossover control in meiosis. *eLife*, 7, e30789. https://doi.org/10.7554/eLife.30789

1018

1019   Zhang, L., Stauffer, W. T., Wang, J. S., Wu, F., Yu, Z., Liu, C., Kim, H. J., & Dernburg, A. F. (2023).

1020   Recruitment of Polo-like kinase couples synapsis to meiotic progression via inactivation of CHK-2.

1021   *eLife*, 12, e84492. https://doi.org/10.7554/eLife.84492

1022

1023   Zhang, L., Stauffer, W. T., Zwicker, D., & Dernburg, A. F. (2021). Crossover patterning through kinase-

1024   regulated condensation and coarsening of recombination nodules. [Preprint]. *bioRxiv*.

1025   https://doi.org/10.1101/2021.08.26.457865

1026

Figure Legends

Figure 1. PCH-2 controls the number and distribution of crossovers in similar patterns on multiple chromosomes. Genetic analysis of meiotic recombination in wildtype and *pch-2* mutants. DCO indicates double crossovers. Physical and genetic maps of Chromosome I, III, IV and the X chromosome are depicted to scale. Genetic distance is shown in centimorgans. A * indicates a p-value < 0.05, a ** indicates a p value < 0.01 and a *** indicates p-value < 0.001.

Figure 2. PCH-2 prevents exogenous double strand breaks from becoming crossovers in early meiotic prophase. A. Illustration of the irradiation experiments in control and *pch-2* mutants. Box indicates late pachytene, the area where GFP::COSA-1 foci are analyzed. **B.** Meiotic nuclei in control animals and *pch-2* mutants 8 hours post irradiation stained for DAPI (magenta) and GFP::COSA-1 (green). Scale bar is 4 um. **C.** Fraction of meiotic nuclei with less than 6, 6, or greater than 6 GFP::COSA-1 foci in control animals(yellow) and *pch-2* mutants (blue) mutants 8 hours post irradiation. **D.** Meiotic nuclei in control animals and *pch-2* mutants 24 hours post irradiation with DAPI (magenta) and GFP::COSA-1 (green). **E.** Fraction of meiotic nuclei with less than 6, 6, or greater than 6 GFP::COSA-1 foci in control animals and *pch-2* mutants 24 hours post irradiation. A *** indicates p-value < 0.001, and a **** indicates p-value < 0.0001.

Figure 3. PCH-2 prevents SPO-11-induced double strand breaks from becoming crossovers in early meiotic prophase. A. Illustration of the SPO-11 depletion experiment to assay GFP::COSA-1 and bivalents in control animals and *pch-2* mutants at different timepoints of auxin treatment. Each timepoint indicates when SPO-11 is depleted in the germline with auxin induced degradation. Boxes indicate late pachytene, the area where GFP::COSA-1 foci are analyzed, and diakinesis, where bivalents are analyzed. **B.** Representative images of meiotic nuclei in control animals and *pch-2* mutants treated with auxin for 20 hours, stained for DAPI (magenta) and GFP::COSA-1 (green). Scale

1054   bar is 5 um. **C.** Number of GFP::COSA-1 foci in meiotic nuclei at different timepoints of auxin

1055   treatment in control (blue) and *pch-2* mutants (yellow). Error bars represent standard error of the

1056   mean (SEM). **D.** Oocytes from control animals and *pch-2* mutants stained for DAPI (magenta). Scale

1057   bar is 4um. **E**. Number of DAPI stained bodies in meiotic nuclei at different timepoints of auxin

1058   treatment in control animals and *pch-2* mutants. Error bars represent SEM and a * indicates p-value <

1059   0.05 and a *** indicates p-value < 0.001.

1060

1061   **Figure 4. PCH-2 is required for timely loading and removal of MSH-5 on meiotic chromosomes**

1062   **through its regulation of HIM-3. A.** Representative images of nuclei in different stages of meiotic

1063   prophase in control animals and *pch-2* mutants stained for DAPI (magenta) and GFP::MSH-5 (green)

1064   Scale bar is 5 um. **B.** Scatter plot showing average GFP::MSH-5 foci per row of germline nuclei in

1065   control animals (yellow) and *pch-2* mutants (blue) from the transition zone to late pachytene,

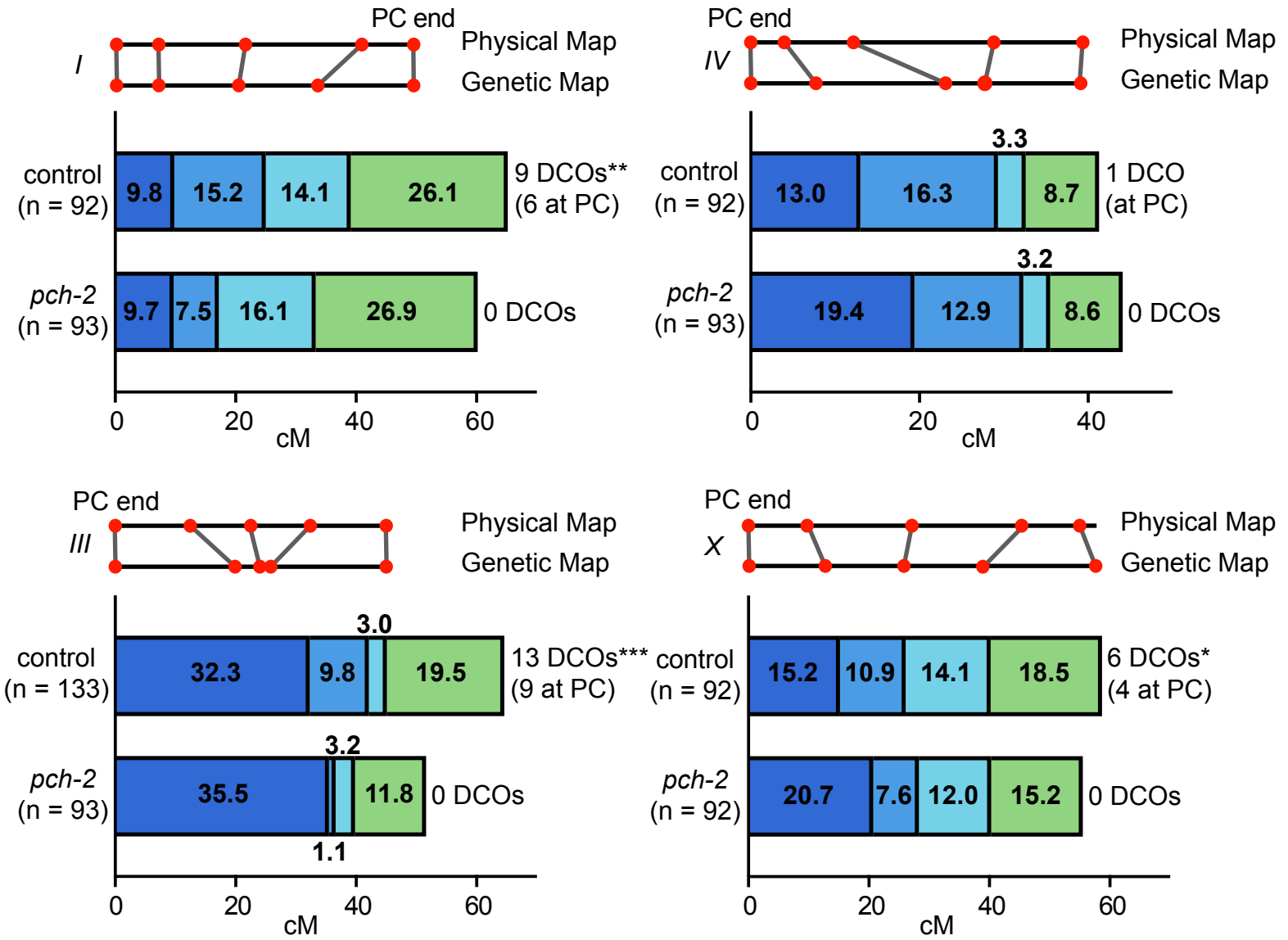1066   normalized to 100. The line represents a rolling average of four rows. **C.** Representative images of

1067   nuclei in different stages of meiotic prophase in *him-3^{R93Y}* mutants (left) and *pch-2;him-3^{R93Y}* double

1068   mutants (right), stained for DAPI (magenta) and GFP::MSH-5 (green). **D.** Scatter plot showing

1069   average GFP::MSH-5 foci per row in *him-3^{R93Y}* (brown) and *pch-2;him-3^{R93Y}* mutants (pink) from the

1070   transition zone to late pachytene, normalized to 100. The line represents a rolling average of four

1071   rows. Similar data is provided for a control germline (opaque yellow) for comparison. **E.**

1072   Representative images of meiotic nuclei in control animals and *pch-2* mutants stained for DAPI (blue),

1073   GFP::MSH-5 (green), and OLLAS::COSA-1 (red). Yellow circles indicate GFP::MSH-5 without

1074   OLLAS::COSA-1. Scale bar is 4um. **F.** Scatter plot showing average GFP::MSH-5 (green) and

1075   OLLAS::COSA-1 (red) foci per row in the last five rows of the germline in control animals and *pch-2*

1076   mutants. The line represents a rolling average of 2 rows. **G.** Swarm plot showing number of

1077   GFP::MSH-5 foci in control (yellow) and *pch-2* mutant (blue) nuclei with less than 6 OLLAS::COSA-1

1078   foci (left) and 6 OLLAS::COSA-1 foci (right). Error bars represent SEM. A * indicates a p-value less

1079   than 0.05 and a ** indicates a p-value < 0.01.

1080

Figure 5. PCH-2 is removed when crossovers are designated. A. Representative images of

meiotic nuclei in *dsb-2* animals 24 hours post L4 and 48 hours post L4 stained for DAPI (magenta),

PCH-2 (red), and GFP::COSA-1 (green). Scale bar is 4 um. **B.** Stacked histograms showing

percentage of PCH-2 positive and negative nuclei with (lime) and without (dark green) GFP::COSA-1

foci in *dsb-2* mutants at 24 hours post L4 and 48 hours post L4. **C.** Representative images of meiotic

nuclei in *dsb-2::AID* and *dsb-2::AID;pch-2* mutants treated with auxin and stained for DAPI (magenta)

and GFP::COSA-1 (green). Scale bar is 5 um. **D.** Swarm plot showing the number of GFP::COSA-1

foci in *dsb-2::AID* (gray) and *dsb-2::AID;pch-2* (lemon) mutants when treated with ethanol or auxin.

Error bars represent the SEM. A ** indicates a p-value less than 0.01 and a **** indicates p-value <

0.0001.

Figure 6. PCH-2 and high CHK-2 activity control the fate of early double strand breaks. A.

Illustration of CHK-2 activity in wildtype and *syp-1^{T452A}* germlines. **B.** Representative images of meiotic

nuclei late pachytene in *syp-1^{T452A}* and *pch-2;syp-1^{T452A}* mutants stained for DAPI (magenta) and

GFP::COSA-1 (green). Scale bar is 5 um. **C.** Swarm plot showing number of GFP::COSA-1 foci in

control animals (blue), *pch-2* (yellow), *syp-1^{T452A}* (maroon), and *pch-2;syp-1^{T452A}* (light blue) mutants.

Error bars represent SEM. **D.** Oocytes from *syp-1^{T452A}* and *pch-2;syp-1^{T452A}* mutant worms stained for

DAPI (magenta). Scale bar is 4um. **D.** Swarm plot showing number of DAPI stained bodies in control

animals (blue), *pch-2* (yellow), *syp-1^{T452A}* (maroon), and *pch-2;syp-1^{T452A}* (light blue) mutants. Error

bars represent SEM. A **** indicates p-value < 0.0001.

Figure 7. PCH-2 remodels HIM-3 to disassemble crossover-eligible intermediates, controlling

crossover distribution and number. A. Model for how *pch-2* and *him-3^{R93Y}* mutations genetically

interact to affect the progression of meiotic recombination. HIM-3 adopts the closed conformation

upon binding an interacting protein with a closure motif and its conversion to the extended

conformation is facilitated by PCH-2's remodeling of its HORMA domain. **B.** Model for how PCH-2 and

HIM-3 progressively implement meiotic recombination during different stages of meiotic prophase.

1108

1109 **Supplemental Figure 1: PCH-2 does not regulate GFP::MSH-5 loading and removal through**

1110 **HTP-3. A.** Representative images of nuclei in different stages of meiotic prophase in *htp-3^{H96Y}* and

1111 *pch-2; htp-3^{H96Y}* mutants stained for DAPI (magenta) and GFP::MSH-5 (green) Scale bar in all images

1112 is 5 um. **B.** Scatter plot showing average GFP::MSH-5 foci per row of germline nuclei in *htp-3^{H96Y}*

1113 (blue) and *pch-2; htp-3^{H96Y}* (green) mutants from the transition zone to late pachytene, normalized to

1114 100. The line represents a rolling average of four rows.

1115

1116 **Supplemental Figure 2: *pch-2* meiotic nuclei with elevated numbers of GFP::MSH-5 foci show**

1117 **defects in crossover assurance.** Gray scale images of control and *pch-2* mutant nuclei stained for

1118 DAPI, GFP::MSH-5 and OLLAS::COSA-1. Scale bar in image is 4 um.

1119

Figure 1

Figure 2

A

8 Hours

24 Hours

B

8 Hours Post Irradiation

control

pch-2

DAPI
COSA-1

C

8 Hours Post Irradiation

control
pch-2

Fraction of Meiotic Nuclei

***

Number of COSA-1 Foci

D

24 Hours Post Irradiation

control

pch-2

E

24 Hours Post Irradiation

Fraction of Meiotic Nuclei

****   ****

Number of COSA-1 Foci

Figure 3

Figure 4

Figure 5

Figure 6

A

wildtype

$syp\text{-}1^{T452A}$

CHK-2 Activity
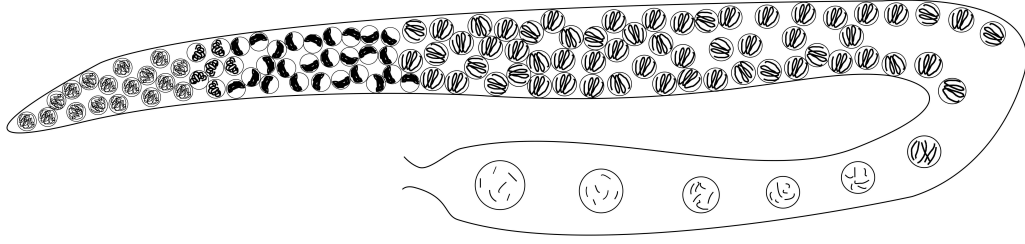
B
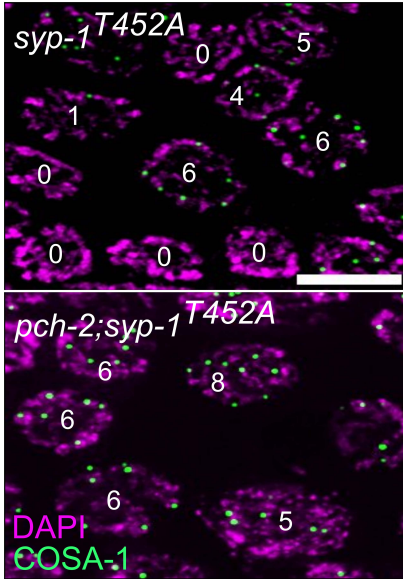
C
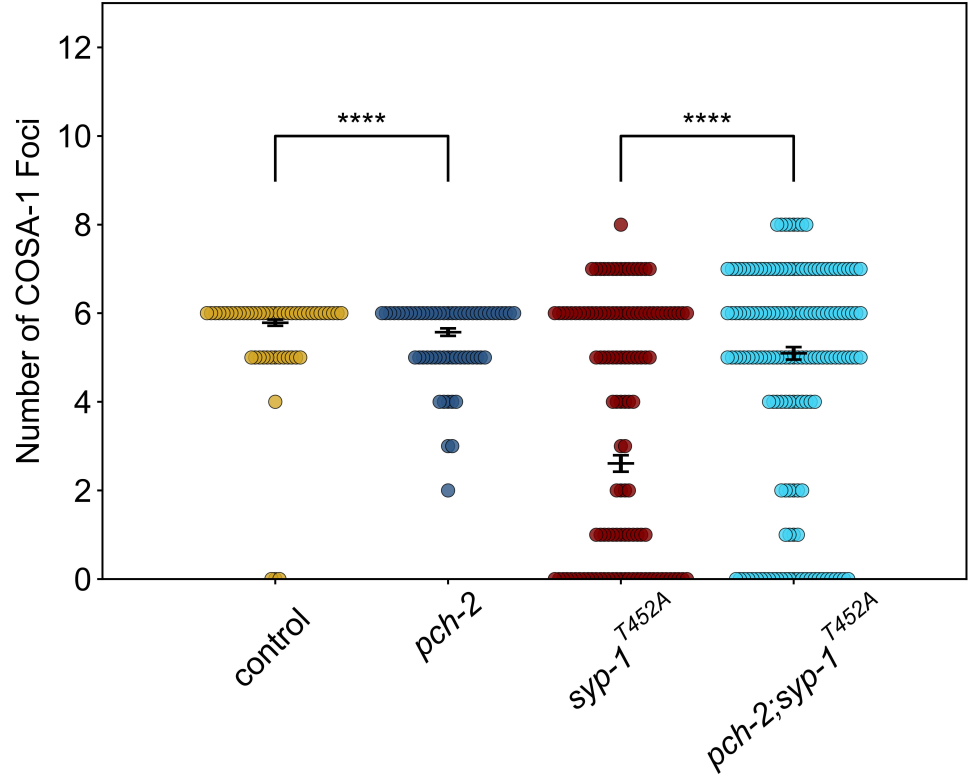
D
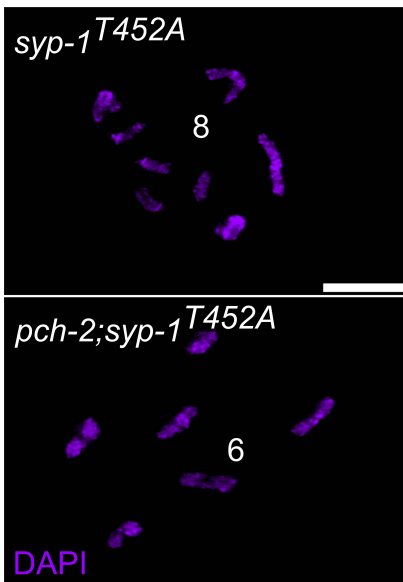
E
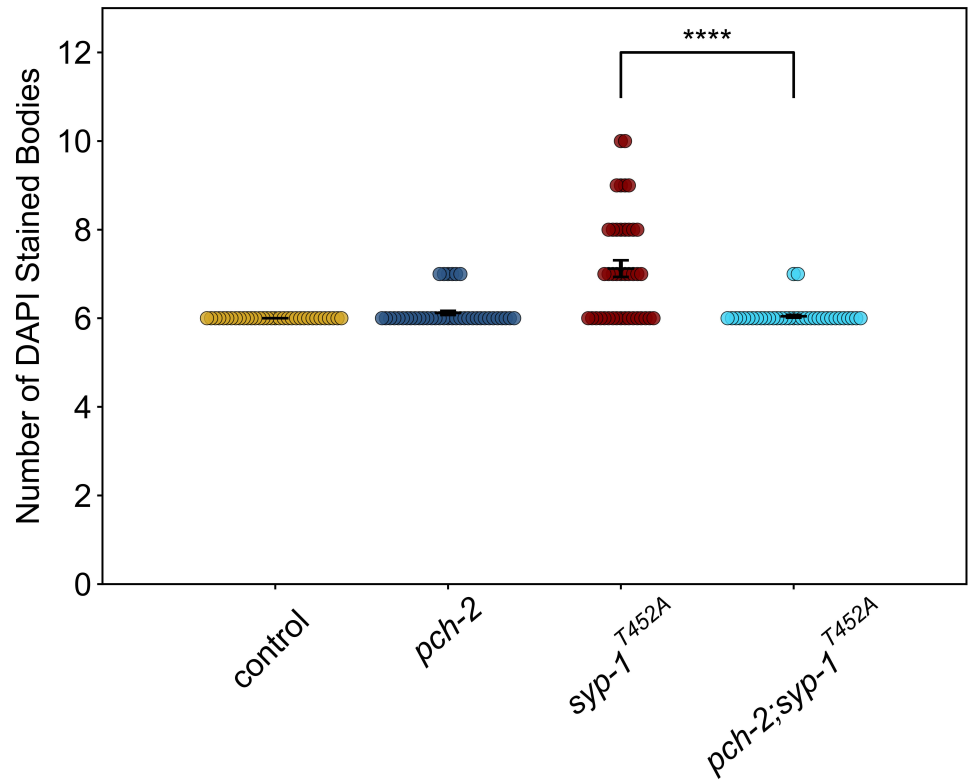
Figure 7

A **wildtype:**

closure motif

PCH-2

extended HIM-3 ⇌ closed HIM-3

pch-2 mutants:

closure motif

extended HIM-3 → closed HIM-3

him-3^{R93Y} mutants:

closure motif

PCH-2

extended HIM-3 ⇌ closed HIM-3

pch-2;him-3^{R93Y} mutants:

closure motif

extended HIM-3 ⇌ closed HIM-3
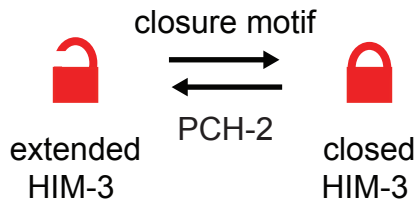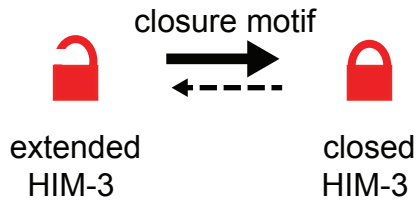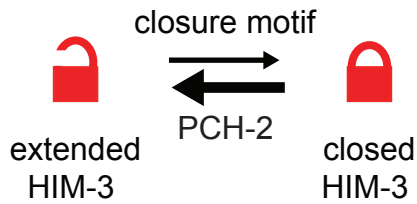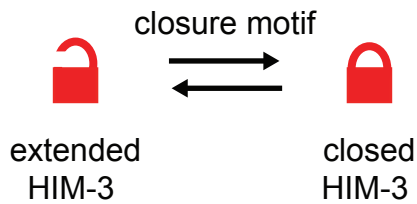
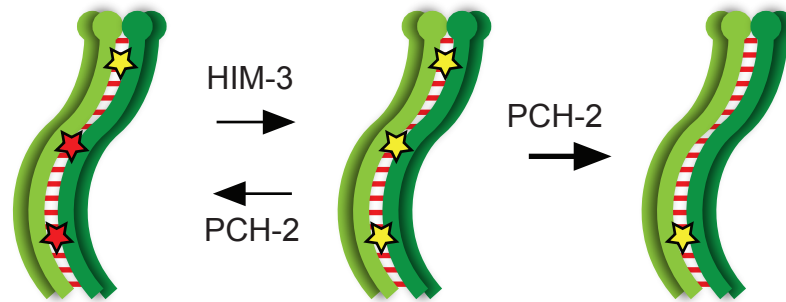**B** **Leptotene/Zygotene:**
high CHK-2 activity
PCH-2 present as foci
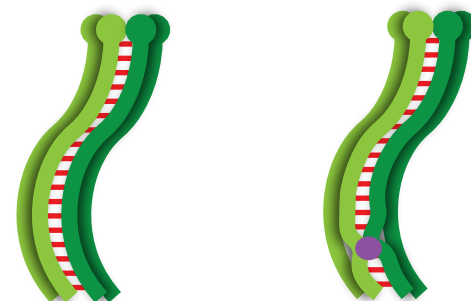on meiotic chromosomes

HIM-3
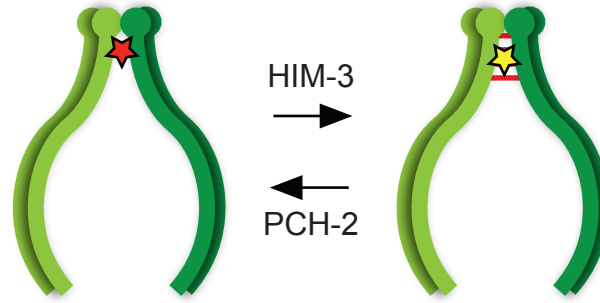
PCH-2

Mid-pachytene:
intermediate CHK-2 activity
PCH-2 localizes to
the synaptonemal complex

HIM-3

PCH-2

PCH-2

Late-pachytene:
no CHK-2 activity
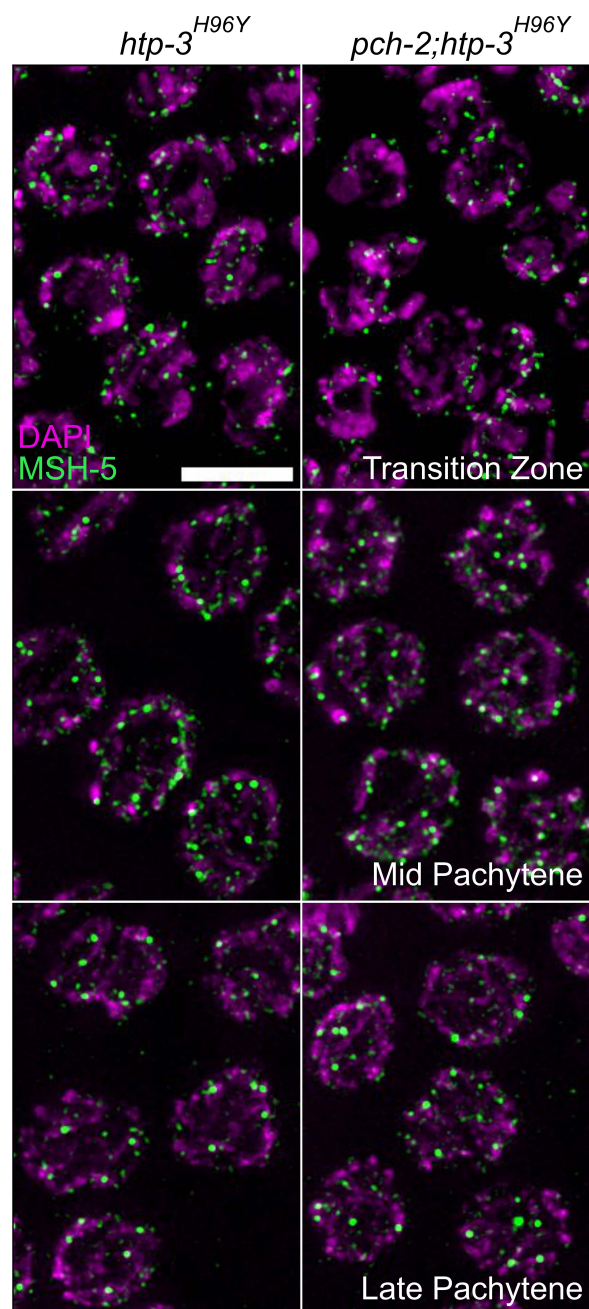PCH-2 absent
crossover designation

no PCH-2

no crossover

⭐ Double strand break

⭐ Crossover-eligible intermediate

● Crossover

Supplementary Table 1: Single nucleotide polymorphisms used for recombination assay
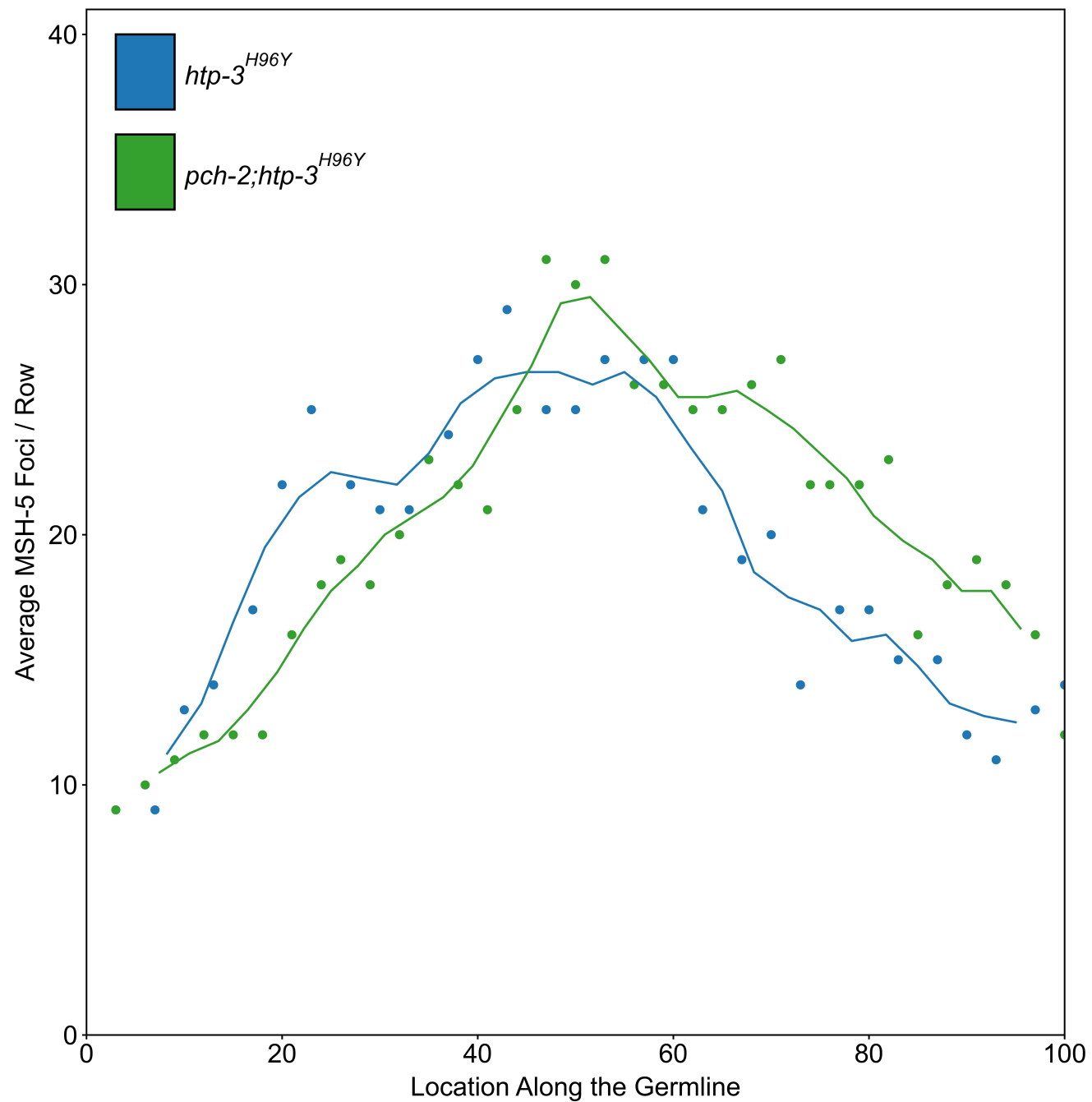
Chromosome and SNP	Primer Name	Primer Genetic Location	Primer Sequence FOR	Primer Sequence REV	Restriction enzyme	N2	HI
IA	F56C11	-19	ATGCCAGTGATAAGGAACGG	TCACATCCCTTGTCGATGAA	DraI	354, 146	500
IB	Y71G12	−12.3	GACAATGACCAATAAGACG	GATCCGTGAAATTGTTCCG	BsrI	440, 125	364, 125, 76
IC	K04F10	0.9	ATCATTCTCCAGGCCACGTTAC	CTGAACTAGTCGAACAAACCCC	NdeI	594	300, 294
ID	T07D10	13.6	CTTGGTGTGGGGAGAGTATAGG	TTTGTCCGGATTGACTCTGC	Sau3AI	303, 63	207, 96, 63
IE	ZK909	28.8	CACAAGTGGTTTGGAAGTACCG	CAACAAAGGGATAGATCACGGG	HindIII	450	236, 214
IIIA	pkP3081	-26.98	AGCAAGAATGAGCCGATTG	GTCGGCCGTTTTCAAATAACTG	TaqI	222, 149	195, 145, 27
IIIB	pkP3095	-5.12	TCTCGTCAATTGTCGCCTG	TTATTTGCAATCCAACGGC	ApoI	308	168, 140
IIIC	pkP3101	-0.9	CCAAGTGCAAACTATGGTGC	ATAAACAATTTCAGTGCCGC	HinfI	495	282, 213
IIID	pkP3035	0.9	CGTAAACTACCAAACTCGGTG	GGTCTACTACAACTATACAGGC	Eco0109I	732	419, 313
IIIE	pkP3080	21.29	CGGTGGTGGTAAAAGTGTAAC	CAACATTCAGGCTGTGCTTTCC	Hpy188III	365, 76, 68, 35	241, 124, 76, 68, 35
IVA	F56B3	-24	TGATGGTGTGTCTGCGTACC	AGAGCTGGAGAGCACGGATA	DraI	301, 128, 71	429, 71
IVB	F52C12	−14.9	ACATTTAGTCACGCGTAGGG	GCCCGAATCTAGCACATAAG	HpaII	191, 137, 22	328, 22
IVC	B0273	1.8	AATACAGCAGTCGTTCCGTTC	TGAACTTCATGAACCAGCTTG	DraI	288, 144	432

IVD	K10D11	6.7	GATTATTT CAGAGGA GCAGAGC	CATAGCAC GTGGAATA ACCAC	HindIII	420	245, 175
IVE	T02D1	16.8	TGCTTAA AGTCATC GTGTCCA C	TGTAAACC GTATCGAA TCCGAC	EarI	174, 235	408
XA	pkP6139	-19.97	AAGAGTG AACCTTT TCCGTGA G	TGATGCAA TTTATACA CACGCC	MseI	401, 31	279, 122, 31
XB	pkP6120	-10.46	TCGTGGC ACCATAA AAGTG	GATTCAGA TCAAACAG AGGTGG	DraI	243	128, 115
XC	pkP6157	-0.14	GGGGTAT AATGAAC CAACCTG	TGTAGGAA CCGTTTGT TTCTTC	ApoI	261, 48	150, 111, 48
XD	pkP6161	9.38	ATCGACC CCAACAA TGCAC	TCCGTCAT CCAAATCT CCG	AseI	542	287, 255
XE	pkP6170	24.07	CGCTGTC ACAATCT CTAAAAT G	AAACCCTC CCCACTTT GTTGTC	ApoI	249, 118, 56	197, 118, 56, 52

A

| $htp\text{-}3^{H96Y}$ | $pch\text{-}2;htp\text{-}3^{H96Y}$ |

DAPI
MSH-5

Transition Zone

Mid Pachytene

Late Pachytene

B

$htp\text{-}3^{H96Y}$

$pch\text{-}2;htp\text{-}3^{H96Y}$

Average MSH-5 Foci / Row

Location Along the Germline

A