DNA damage response clamp loader Rad24(Rad17) and Mec1(ATR) kinase have distinct functions in regulating meiotic crossovers

Miki Shinohara*, †, ‡, †, Douglas K. Bishop‡ and Akira Shinohara*, †

*Institute for Protein Research, Osaka University, Osaka, 565-0871, JAPAN,

†School of Agriculture, Kindai University, Nara, 631-8505, JAPAN

‡Department of Radiation Oncology/Department of Molecular Genetics and Cell Biology, University of Chicago, Chicago, IL 60637, USA

ORCID: 0000-0001-5061-8085 (D.K.B), 0000-0003-4207-8247(A.S.)
Running title: Roles of Rad24 and Mec1 in CO control

Keywords: crossovers, crossover interference, Rad24, Mec1, meiosis

¹Correspondence:
Akira Shinohara
Institute for Protein Research,
Osaka University
3-2 Yamadaoka, Suita, Osaka, 565-0871, Japan
Tel: +81-6-6879-8624
Fax: +81-6-6879-8626
E-mail: ashino@protein.osaka-u.ac.jp
ORCID: 0000-0003-4207-8247

Miki Shinohara
School of Agriculture,
Kindai University

3327-204 Nakamachi, Nara, 631-8505, Japan

Tel: +81-742-43-6518

Fax: +81-742-43-8976

E-mail: mikis@nara.kindai.ac.jp
Abstract

Crossover (CO) recombination is essential for chromosome segregation during meiosis I. The number and distribution of COs are tightly regulated during meiosis. CO control includes CO assurance and CO interference, which guarantee at least one CO per a bivalent and evenly-spaced CO distribution, respectively. Previous studies showed the role of DNA damage response (DDR) clamp and its loader in efficient formation of meiotic COs by promoting the recruitment of a pro-CO protein Zip3 and interhomolog recombination, and also by suppressing ectopic recombination. In this study, by classical tetrad analysis of *Saccharomyces cerevisiae*, we showed that a mutant defective in the *RAD24* gene (*RAD17* in other organisms), which encodes the DDR clamp loader, displayed reduced CO frequencies on two shorter chromosomes (III and V) but not on a long chromosome (chromosome VII). The residual COs in the rad24 mutant do not show interference. In contrast to the rad24 mutant, mutants defective in the ATR kinase homolog Mec1/Esr1,
including a mecl null and a mecl kinase-dead mutant, show little or no
defect in CO frequency. On the other hand, mecl COs show defects in
interference, similar to the rad24 mutant. Moreover, CO formation and its
control are implemented in a chromosome-specific manner, which may
reflect a role for chromosome size in regulation.
Introduction

Homologous recombination between two DNAs generates both crossover (CO) and non-crossover (NCO) products. CO recombinants display reciprocal exchange of the flanking DNA regions while NCO display uni-directional transfer of genetic information from one to the other parental DNAs with flanking regions remaining in their parental configuration. Meiotic recombination is regulated to promote the formation of COs while mitotic recombination is regulated to suppress them (HEYER et al. 2010; HUNTER 2015). During meiosis COs play an essential role in correct segregation of homologous chromosomes during meiosis I (MI) for generation of gametes. As a consequence of their importance for chromosome segregation, the frequency and distribution of COs is strictly regulated during meiosis via a set of mechanisms referred to collectively as crossover control (HUNTER 2015; SAITO AND COLAIACOVO 2017).

Meiotic CO formation is initiated by the introduction of DNA double-strand breaks (DSBs) (KEENEY 2001). Nucleolytic processing of DSB ends generates tracts of single-stranded DNA (ssDNA) that are substrates for the formation of
nucleoprotein filaments by the strand exchange protein Dmc1 which then carries out a search for regions of homologous duplex DNA (Bishop et al. 1992). Invasion of the ssDNA into the homologous duplex results in the formation of an unstable displacement-loop (D-loop) (Hunter and Kleckner 2001). Then, a subset of D-loops is converted into a “stable” D-loop intermediates, that are destined to generate COs. Such CO intermediates are referred to as a single-strand invasion (SEI) (Hunter and Kleckner 2001). Further processing of SEIs produces double Holliday junction (dHJ) intermediates (Schwacha and Kleckner 1995). SEIs and dHJs are preferentially formed between homologous chromosomes rather than between sister chromatids (Schwacha and Kleckner 1994; Schwacha and Kleckner 1997). This is referred as to “interhomolog (IH) bias”. Finally, dHJs are biased to give rise to CO rather than NCO products (Allers and Lichten 2001; Hunter and Kleckner 2001). NCO recombinants are usually produced through the synthesis-dependent strand annealing pathway that does not form stable SEIs and dHJs (Allers and Lichten 2001; Hunter and Kleckner 2001; Borner et al. 2004; McMahill et al. 2007).
CO formation during meiotic prophase I is fostered by a group of proteins called ZMM (Zip, Mer, Msh) or SIC proteins (Synaptic Initiation Complex), hereafter ZMM for simplicity (CHUA AND ROEDER 1998; AGARWAL AND ROEDER 2000; BORNER et al. 2004; SHINOHARA et al. 2008). ZMMs include Zip1, Zip2, Zip3, Mer3, Msh4, Msh5, Spo22/Zip4 and Spo16. Msh4-Msh5 and Zip2-Zip4-Spo16 sub-complexes bind to branched DNAs (SNOWDEN et al. 2004; ARORA AND CORBETT 2018; DE MYJT et al. 2018). Mer3 encodes a 5’-3’ DNA helicase (NAKAGAWA AND OGAWA 1999; NAKAGAWA et al. 2001). Zip3 is a putative SUMO/Ubiquitin E3 ligase (AGARWAL AND ROEDER 2000; PERRY et al. 2005; CHENG et al. 2006). ZMM proteins localize on meiotic chromosomes during prophase-I, which can be detected cytologically as immunostaining foci. Zip3, which is bound to DSB sites, promotes local assembly of other ZMM proteins (SHINOHARA et al. 2008; SERRENTINO et al. 2013). ZMM proteins also promote local initiation of synaptonemal complex (SC), (SYM et al. 1993; STORLazzi et al. 1996), and coordinate SC formation with recombination (BORNER et al. 2004; SHINOHARA et al. 2008). ZMM proteins not only promote CO formation, but are
also required for the normal distribution of meiotic COs (SYM AND ROEDER 1994; CHUA AND ROEDER 1998; AGARWAL AND ROEDER 2000; NOVAK et al. 2001; SHINOHARA et al. 2008).

Meiotic CO control involves three types of regulation. First, CO interference, which results in more even spacing of COs than expected at random (MULLER 1916). Second, CO assurance distributes crossovers among chromosomes such that each chromosome receives at least one crossover, even when the average number of COs per chromosome in a single nucleus is low (JONES 1987; BISHOP AND ZICKLER 2004). Third, meiotic cells display “CO homeostasis”, a mechanism that buffers against low levels of dedicated CO intermediates by maintaining normal frequencies of COs at the expense of NCOs (MARTINI et al. 2006; LAO et al. 2013). Crossover frequency and control is also regulated in a chromosome-size (KABACK et al. 1992; KABACK et al. 1999) and also in per-nuclear basis (WANG et al. 2019). Cytological studies showed that the spacing of ZMM foci such as Zip3 indicates that crossover interference is established in zmm mutants, but that the sites designated to form COs are not
maintained and many sites initially designated to become COs form NCOs instead, suggesting that ZMM is required for the maintenance of the class of COs that display CO interference (type 1 COs) (FUNG et al. 2004; ZHANG et al. 2014a). ZMM-dependent COs represent about 50-70% of the total number of COs observed in WT cells. The COs that remain in zmm mutants (type 2 COs) do not display interference. Importantly, topoisomerase II is necessary for interference between ZMM foci, indicating topoisomerase II is required to establish the regulated distribution of COs (ZHANG et al. 2014b). CO interference is also regulated by proteins, which directly catalyze interhomolog recombination during meiosis (SHINOHARA et al. 2003b).

DNA damage response (DDR) proteins play an important role in the response to DNA damage in both mitotic and meiotic cells (HOCHWAGEN AND AMON 2006). During mitotic DDR in S. cerevisiae, the Rad24-RFC clamp-loader complex (the Rad17-RFC complex in other organisms) promotes recruitment of the Ddc1-Mec3-Rad17 clamp complex (“9-1-1” [Rad9-Rad1-Hus1] complex in other organisms) to tracts of ssDNA (MAJKA AND BURGERS 2003; MAJKA et al.
2006). Mec1(ATR) kinase is recruited on replication protein A (RPA)-coated ssDNAs through Ddc2(ATRIP) protein (Zou and Elledge 2003). Mec1 recruitment and activation are partly dependent on the clamp (Majka et al. 2006).

In meiosis, as in mitosis, DDR proteins can induce delays in the entry into the first meiotic division (MI) when meiotic recombination is defective (Lydall et al. 1996; San-Segundo and Roeder 2000). This delay during meiosis prophase I is imposed at the exit of pachytene, not at the onset of anaphase I (Subramanian and Hochwagen 2014). Cooperation of the DDR clamp and Mec1 activates a meiosis-specific CHK2 homolog, Mek1 kinase, which in turn relays signals to downstream targets (Hollingsworth and Gaglione 2019). One of Mek1 targets is Ndt80 transcriptional activator, which promotes pachytene exit by regulating the expression of genes required for late meiosis (Hollingsworth and Gaglione 2019). In addition to providing a means to regulate meiotic progression when recombination is incomplete, the DDR proteins work directly in meiotic recombination (Grushcow et al. 1999; Thompson and Stahl 1999; Shinohara et al. 2003a). The 911 clamp and Mec1 suppress non-allelic (ectopic)
recombination (Grushcow et al. 1999; Thompson and Stahl 1999; Shinohara and Shinohara 2013). Furthermore, Mec1 promotes IH bias, presumably via its kinase activity, although the relevant substrates have yet to be identified, but given that Mec1 activates the down-stream kinase Mek1, and Mek1 is required for homolog bias (ref Kim/Kleckner-I think), it is likely that Mec1’s role in bias involves Mek1 activation.

Recently, we showed that 911 clamp, but not Mec1, promotes the loading of Zip3, thereby regulating ZMM assembly through direct protein interaction (Shinohara et al. 2015). Consistent with this, DNA analysis showed that the 911 and rad24 mutants decreases the frequency of COs about 2-fold, as do zmm mutants (Grushcow et al. 1999; Shinohara et al. 2003a). Moreover, the rad24 mutant is defective in IH bias (Shinohara et al. 2015). This particular feature of rad24 is reminiscent of mutants lacking a functional RAD51 gene; RAD51 encodes a RecA homolog (Shinohara et al. 1992), that acts as an regulatory protein for the meiosis-specific RecA homolog Dmc1 during meiosis; Rad51 promotes Dmc1-mediated IH recombination (Schwacha and Kleckner 1997;
Over-expression of *RAD51* and its partner

*RAD54* can suppress the reduced spore viability and X-ray sensitivity of *rad17* and *rad24* mutants (Shinozawa et al. 2003a). Earlier studies in *Drosophila* showed that a mutant defective in the DDR kinase ATR homolog, *mei-41*, change the frequencies and distribution of meiotic COs along chromosomes (Carpenter and Baker 1982; Brady et al. 2018). However, the possible role of budding yeast DDR proteins in CO control has not been addressed.

In this paper, we provide genetic evidence for a novel role of the DDR clamp loader, Rad24, in efficient formation of meiotic COs on chromosomes *III* and *V*, which are relatively short chromosomes, but not on chromosome *VII*, one of the longest chromosomes. The COs that occur in the *rad24* mutant show little or no positive interference. Our results support the hypothesis that the clamp and clamp loader proteins promote interfering COs by promoting the recruitment of ZMM proteins to recombination sites. On the other hand, we find, although the *mec1* mutant displays reduced CO frequency on chromosome *III*, it displays increased CO frequency on chromosome *VII*. As with the *rad24* mutant, COs
formed in the absence of Mec1 kinase activity display little if any interference. These observations suggest that Mec1 kinase regulates CO formation differentially from the DDR clamp loader. Mec1 may phosphorylate a novel protein, which is specifically involved in CO control, rather than being required for CO formation.

Materials and Methods

Strains and plasmids

All strains described here are derivatives of SK1 diploids except S2921 and MSY172, which are congenic strain (SYM AND ROEDER 1994). Strain genotypes are given in Table S1.

Analyses of meiotic recombination

Time-course analyses of DNA events in meiosis were performed as described previously (STORLAZZI et al. 1995; SHINOHARA et al. 1997). Genomic DNAs
prepared were digested with *Mlu*I, *Bam*HI and *Xho*I (for heteroduplex) and *Pst*I (for meiotic DSB). Probes for Southern blotting were Probe “155” for HD and Probe 291 for DSB detection as described in (STORLAZZI et al. 1995). Image gauge software (Fujifilm Co. Ltd., Japan) was used for quantification for bands of R1, R3 and DSB I.

**Genetic analysis of meiotic recombination**

Genetic distances between markers and interference were analyzed as described before (SHINOHARA et al. 2003b; SHINOHARA et al. 2008).

Parental haploid strains were mated for 3 h on YPAD plates at 30°C and transferred onto SPM plates. After incubation at 30°C for 48 h, tetrads were dissected onto YPAD and incubated for 2 days. Genotyping was carried out as described (SHINOHARA et al. 2003a). In order to avoid aberrant clones (e.g. those containing mitotic crossovers), at least four independent crosses were carried out.

For interference analysis and genetic distance calculations, tetrads with
non-Mendelian segregation of a diagnostic marker were excluded from the analysis. Map distances were determined using the standard mapping equation 

\[ [cM] = \frac{100}{2(TT+6NPD)} \div (PD+TT+NPD) \]

Standard errors were calculated using the Stahl Lab online tool (http://www.molbio.uoregon.edu/~fstahl). Interference values are expressed as the NPD ratio; the fraction of tetrads expected to be NPDs was determined from the Papazian equation: 

\[ NPD_{ex} = \frac{1}{2} \left[ 1 - TT \cdot (1-3T/2)^{2/3} \right] \]

where TT is the proportion of tetratypes observed (PAPAZIAN 1952). Data sets were analyzed using chi-square test.

To measure coincident double crossovers in adjacent intervals, the frequencies of tetrads with recombination in each of the two intervals were determined by summing TT and NPD tetrads for those intervals and dividing by the total number of tetrads (SHINOHARA et al. 2003b). The expected frequency of coincident recombination is given by the product of two single-interval frequencies.

**Statistical analysis**
Graphs were prepared using Microsoft Excel and GraphPad Prism 7.

Datasets were compared using the Mann-Whitney U-test. $\chi^2$-test was used for proportion. Multiple test correction was done with Bonferroni’s correction. *, **, and *** show $P$-values of <0.05, <0.01 and <0.001, respectively.

**Data availability**

Strains and plasmids are available upon request. The authors affirm that all data necessary for confirming the conclusions of the article are present within the article, figures, and tables.

**Results**

**The rad24 mutant is defective in crossover formation and interference**

Physical analyses have shown that the 9-1-1 clamp and its loader mutants in budding yeast *Saccharomyces cerevisiae* reduce crossover formation at a recombination hotspot on chromosome III (see below) (GRUSHCOW et al. 1999;
SHINOHARA et al. 2003a). To further characterize checkpoint mutant defects in crossover formation and control, we carried out tetrad analysis of meiotic recombination in the rad24 mutant. For the analysis, we used markers on chromosome V in strains congenic with SK1 (Figure 1A) (SYM AND ROEDER 1994; SHINOHARA et al. 2003b). We dissected 1196 and 8060 tetrads for wild type and the rad24 strains, respectively. Consistent with previous reports (LYDALL et al. 1996; SHINOHARA et al. 2003a), the rad24 mutant shows 32% spore viability and more tetrads having 0-, 2- and 4-viable spores per tetrad than those having 1- or 3-viable spores (Figure S1A); this pattern is consistent with loss of viability resulting from a high level of chromosome non-disjunction during the first meiotic division. Only about 1 in 6-7 tetrads formed by rad24 mutants contained 4 viable spores. When genetic distances between markers on the chromosome are measured in 4-viable-spore tetrads, the rad24 mutant reduces the distance of three intervals to about half of those in wild type (Figure 1, B and C and Table 1). Frequencies of non-Mendelian segregation for all four loci were also decreased 1.3 to 16-fold in the mutant (Table 2). The ability of a CO to interfere with
coincident COs was determined by analysis of the ratio of observed to expected non-parental di-type (NPD) tetrads using the method of Papazian (see Materials and Methods). Wild type shows an $\frac{NPD_{\text{observed}}}{NPD_{\text{expected}}}$ (hereafter NPD) ratio of 0.37, 0.32, and 0.24 for the CAN1-URA3, URA3-HOM3 and HOM3-TRP2 intervals, respectively, indicative of CO interference. The $rad24$ mutant exhibits an NPD ratio near unity for three intervals (1.27, 0.87, and 0.8; Figure 2A and Table 1). This is the expected result for mutants defective in forming COs that display interference. These data show a role for Rad24 in controlling the distribution of COs along chromosomes. The defects in crossover formation and control in the checkpoint mutant are similar to those in the $zmm$ core mutants such as $zip1$ (Sym and Roeder 1994).

NPD ratios detect interference between 4-strand double COs in a single interval, but they do not report on the interference that occurs between COs in two different intervals. The coefficient of coincidence [CoC] measures interference between COs in adjacent intervals. Interestingly, when the above tetrad data were analyzed for CoC, we noticed an unusual phenotype in the
rad24 mutant. Because two adjacent intervals used in this study are relatively long, wild type does not show any CO interference between two adjacent intervals (CoC is 0.90 and 0.98 for the CAN1-URA3+URA3-HOM3 and URA3-HOM3+HOM3-TRP2 interval pairs respectively; Figure 2B and Table S1). However, the rad24 mutant displays negative interference; CoC in CAN1-URA3-HOM3 and URA3-HOM3-TRP2 is 1.33 and 1.6, respectively (Figure 2B and Table S2). These are significantly higher than 1 ($P=8.0\times10^{-5}$ for CAN1-URA3+URA3-HOM3 and $1.5\times10^{-5}$ for URA3-HOM3+HOM3-TRP2).

Negative CO interference has been observed for another mutant strain (the dmc1 hed1 double mutant)(LAO et al. 2013), which, like rad24, is defective in interhomolog partner choice. This finding suggests that RAD24 may prevent clustering of COs on chromosome V.

The rad24 mutant is defective in CO interference on chromosomes III and VII

We also analyzed several intervals on a short chromosome (Ch. III) and a long
chromosome (Ch. VII) in a pure SK1 background (HIGASHIDE AND SHINOHARA 2016) (Figure 1A). We dissected 1290 and 12090 tetrads for wild type and the rad24 strains in this background, respectively. The rad24 mutant showed reduced spore viability (31.2%; 97.7% in wild type; Figure S1B) in the pure SK1 background, which is similar to that in the congenic chromosome V-marked strain described above. The rad24 mutant displayed CO frequencies in four intervals (HML-URA3, URA3-LEU2, LEU2-HIS4, and HIS4-MAT) that were roughly half as frequent overall as those in wild type (0.27-0.72 fold reduced; Figure 1, B and C, and Table 3). CO interference, as measured by NPD analysis, was almost compromised in the rad24 mutant (Figure 2A and Table 3). This result is completely consistent with the corresponding data from the analysis of chromosome V in the congenic strain. When the intervals on chromosome VII were analyzed, the rad24 mutant displayed a CO frequency in the CUP2-MET12 interval that was roughly half that in wild-type (Figure 1C and Table 4). On the other hand, the other three intervals; MET13-URA3, URA3-CYH2, and CYH2-ADE6 showed similar CO frequencies in wild-type and rad24. NPD ratios
in three of the four intervals examined (Figure 2A and Table 4); *CUP2-MET12, URA3-CYH2 and CYH2-ADE6* are almost one in the *rad24* mutant, indicating the absence of CO interference. Interference in the *MET13-URA3* was weakened relative to wild type (0.46 versus 0.063), but the data imply significant residual interference. These data suggest a chromosome (or an interval)-specific effect of the *rad24* mutation on CO frequency (see Discussion). We therefore determined CoC for three pairs of adjacent intervals on each chromosome III and VII in wild type (Figure 2B, and Table S3 and S4). With the exception of the *CYH2-TRP5+TRP5-ADE6* interval pair, five pairs showed CoC with less than one (Figure 2B), supporting the presence of CO interference between separated loci in wild type. In the *rad24*, the CoC values from four interval pairs are almost one or more than one. This supports the idea that COs formed in a given interval do not interfere with formation of COs in an adjacent interval in the absence of Rad24.

The *rad24* mutant displayed non-Mendelian segregation frequencies similar to wild-type at four of eight loci examined (Table 5). The other four loci
showed an increase in the frequency of non-Mendelian segregation in the \textit{rad24} mutant. These data indicate that the \textit{rad24} mutant is proficient in NCO formation, but defective in CO formation on chromosome \textit{III} and \textit{VII}.

The \textit{mec1} null mutant is defective in CO interference, but proficient in CO formation on chromosome \textit{V}

Our previous study involving immunostaining of spread chromosomes revealed a difference between the effects of \textit{rad24} and \textit{mec1} mutations on chromosome-associated ZMM complexes, with only \textit{rad24} conferring a defect in ZMM focus formation (Shinohara \textit{et al.} 2015). We therefore analyzed \textit{mec1} mutants in the congenic strain background for CO formation and control on chromosome \textit{V}. The \textit{mec1} deletion mutant was examined in combination with a \textit{sml1-x} mutation, which suppresses the lethality conferred by the \textit{mec1} by increasing levels of ribonucleotides (Zhao \textit{et al.} 1998). The \textit{mec1 sml1-x} double mutant shows 48.0\% spore viability (5570 tetrads dissected), which is significantly higher than seen in the \textit{rad24} mutant, consistent with a previous
Like the \textit{rad24} mutant, the \textit{mec1} deletion mutant produces more 4-, 2- and 0-viable spore asci compared to the number of 3- and 1-viable spore asci (Figure S1A). Surprisingly, in obvious contrast to the \textit{rad24} mutant, the \textit{mec1} mutant displays the same map distance as wild type for one interval on Chromosome \textit{V}, and a greater than wild-type map distance for two intervals (Figure 1C and Table 1). Moreover, the NPD ratio in the \textit{mec1} mutant is essentially unity in two intervals, and is higher than wild type in a third interval (Figure 2A and Table 1). Furthermore, in contrast to the \textit{rad24} mutant, the \textit{mec1} mutant displays higher levels of non-Mendelian segregation than wild type (Table 2). Similar to the results obtained with the \textit{rad24} mutant, the \textit{mec1} mutant displayed negative interference, with CoC values exceeding unity (Figure 2B and Table S2). These findings suggest that \textit{MEC1(ATR)} is not required for formation of normal levels of COs, but rather that the COs formed in \textit{mec1} are distributed abnormally. Thus, the \textit{mec1} mutant differs from the \textit{rad24} mutant with respect to the ability to form normal levels of meiotic COs, but is similar in that it displays defects in positive interference, as well as apparent negative interference of
distant coincident COs (See Discussion).

The *mec1* mutants show chromosome-specific effects on CO formation

We also examined the effect of the *MEC1* deletion on meiotic recombination on chromosomes *III* and *VII* in a pure SK1 background. To rescue the lethality of the *MEC1* deletion, we introduced the *sml1*-x mutation and analyzed COs in 4-viable spore tetrads of *mec1-1 sml1*-x (hereafter, *mec1*). The *mec1* mutant showed reduced spore viability (60.2% in 3827 tetrad dissection) in the pure SK1 background, which is higher than that in the congenic background. On chromosome *III*, the *mec1* mutant reduced CO frequencies relative to wild type in three intervals; the ratio of CO frequency of mutant to wild type was 0.38 for *HML-URA3*, 0.68 for *URA3-LEU2*, and 0.45 for *LEU2-HIS4* (Figure 1, C and D, and Table 3). On the other hand, CO frequency in the *HIS4-MAT* in the *mec1-1* mutant is similar to that in wild type (0.95 fold). These indicate that the *mec1-1* mutant displays significant defects in the frequency of COs on chromosome *III* (Figure 1B). The NPD ratio in two intervals, *URA3-LEU2* and *HIS4-MAT*, was not
significantly different from one in the *mec1-1* mutant (Figure 2A and Table 3),
consistent with the absence of CO interference. The NPD ratio observed in the
*HML-URA3* interval was 2.2, indicating negative interference (It should be noted,
however, that this data set contains only 2 NPDs).

On chromosome *VII*, all four intervals, *CUP2-MET13, MET13-URA3, URA3-CYH2* and *CYH2-ADE6* show significant increase of CO frequencies in the *mec1* mutant compared to wild type; CO frequency ratios were 1.13-1.39 compared to wild type (Figure 1, B-D and Table 4). NPD ratios in the three intervals in the *mec1-1* mutant were all less than one (0.58, 0.57 and 0.85 in *CUP2-MET13, MET13-URA3, URA3-CYH2*, respectively), but significantly greater than those in wild-type. The NPD ratio for the *CYH2-ADE6* interval is about one in the *mec1-1* mutant. These data indicate that Mec1 is partially required for CO interference on chromosome *VII*. With respect to CoC values (Figure 2B and Table S3), *mec1-1* shows the same CoC value for the
*LEU2-HIS4* and *HIS4-MAT* intervals as wild type. The CoC values obtained for
the other three interval pairs examined were significantly higher in *mec1-1* than
those in wild-type, and were close to one. This again supports the role of Mec1 in normal distribution of COs along chromosomes.

For non-Mendelian segregation, the mec1-1 mutant showed increased frequencies at 7 of 8 loci examined on chromosomes III and VII (Table 5). The exception was the HML locus, which was assayed via marker insertion; the mec1 mutation did not show a significant effect on non-Mendelian segregation frequency at that locus.

**Mec1 kinase activity is required for CO interference**

Given that Mec1 belongs to a PI3-kinase family (MAJKA et al. 2006), we wondered whether the kinase activity of Mec1 is required for CO control during meiosis. We introduced the mec1-kd (kinase-dead; D2224A) allele in the pure SK1 background (with sml1-x) with markers on chromosomes III and VII. The mec1-kd mutant showed reduced spore viability (75.5% in 3218 tetrad dissection), which is higher than that in the mec1-1 and mec1 null mutants, suggesting the mec1-kd mutation is a hypomorphic mutant. On chromosome III,
the mec1-kd mutant reduced CO frequencies compared to the wild type in three intervals, with frequency ratios of 0.7 for HML-URA3, 0.85 for URA3-LEU2, and 0.82 for LEU2-HIS4 (Figure 1, C and D, and Table 3). On the other hand, CO frequency in the HIS4-MAT in the mec1-kd mutant is slightly higher than that in wild type (1.2 fold). Compared to the mec1-1 mutant, the mec1-kd mutant showed weaker defects in CO formation. NPD ratios of HML-URA3 and URA3-LEU2 in the mec1-kd were 0.54 and 0.79 respectively, significantly higher than those in wild type, but significantly lower than one (Figure 2A and Table 3). Furthermore, the ratios are significantly lower than those in the mec1-1 mutant. On the other hand, NPD ratio of the HIS4-MAT interval in the mutant is 0.97, indicating a strong defect in interference. Thus, the mec1-kd mutant shows weaker defects in CO formation and interference than mec1-1 mutant. This suggests that there is significant residual CO interference in the mec1-kd relative to mec1-1. This may reflect residual kinase activity or a kinase-independent role of Mec1 in CO interference.

For the intervals on chromosome VII, the mec1-kd mutant showed
increases in the frequency of COs for all intervals (1.17-1.45 fold; Figure 1, C and D, and Table 4), which is similar to the results obtained in the mec1 null mutant. The NPD ratios of all four intervals in the mec1-kd mutant ranged from 0.39 to 0.87 (Table 4). These are values are significantly higher than those in wild type, but lower than those in the null mutant. Thus, the mec1-kd mutant displays weaker defects in CO control than the mec1 null mutant. With respect to CoC values (Figure 2B and Table S3 and S4), among five pairs, the mec1-kd shows the same CoC value for the LEU2-HIS4 and HIS4-MAT intervals as wild type. The CoC values of the HML-URA3: URA3-LEU2 pair of intervals and the URA3-LEU2: LEU2-HIS4 pair of intervals on chromosome III in the mec1-kd mutant are significantly higher than those in the wild-type, which are more than one, again indicating the absence of interference between CO events in adjacent intervals. On chromosome VII, CoC of for the CUP2-MET13:MET13-CYH2 interval pair in the mec1-kd is significantly higher than that in wild type. On the other hand, CoC value of the MET13-CYH2:CYH2-TRP5 intervals in the mec1 mutant is significantly, though modestly, higher than that in wild type. These data
are consistent with the above result that the *mec1-kd* mutant showed a weaker defect in CO interference than the *mec1-1* mutant.

The *mec1-kd* mutation increased the frequencies of non-Mendelian segregation at 7 out 8 loci examined on chromosomes III and VII, with the exception being the *HML* locus (Table 5). This trend is similar to that observed in the null mutant.

**RAD24 functions upstream of ZIP1 in meiotic recombination**

The above genetic analysis showed a similarity between *rad24* and the *zmm* mutants in terms of CO defects. To determine the relationship *DDR* and *ZMM* genes, we carried out DNA analysis. Previous physical analysis showed that the *ZIP1* and the *RAD24* genes work in the same pathway to suppress non-allelic meiotic recombination (Shinohara and Shinohara 2013). We checked whether *ZMM* and *DDR* genes work in the same pathway of recombination by analyzing meiotic recombination products of the *rad24 zip1* double mutant at a DNA level. We examined levels of CO and NCO heteroduplexes (HDs), as well as levels of
ectopic (EC) recombinants at the *HIS4-LEU2* recombination hot spot on a single blot (Figure 3, A and B). The HD assay was used in place of the conventional NCO/CO assay (STORL AZZI et al. 1995) because the HD assay resolves bands specific for EC from all NCO/CO bands (SHINOHARA AND SHINOHARA 2013). Comparison of two phenotypes of the *rad24 zip1* double mutant with those of the corresponding single mutants shows that *rad24* and *zip1* affect meiotic recombination on the same pathway (Figure 3B). First, the *rad24 zip1* double mutant shows elevated levels of EC recombination like *rad24* rather than reduced levels like *zip1* (Figure 3, B and C). This is consistent with our previous study of direct measurement of EC (SHINOHARA AND SHINOHARA 2013). Second, and particularly important to the central conclusion of this study, CO-HD levels in *zip1* are similar to those in the *rad24 zip1* double mutant (Figure 3C). Third, the *rad24 zip1* double mutant shows a decrease in the NCO/CO-HD ratio relative to wild type like the *rad24* single mutant, rather than an increase in the ratio like the *zip1* single mutant. The ratio of NCO/CO-HD is about 1.3 in WT cells. This ratio is reduced to 0.6 in the *rad24* mutant and increased to 3.5 in *zip1*. The
NCO/CO-HD ratio in the rad24 zip1 double mutant is about 0.6, equivalent to the rad24 single mutant. These observations imply that the COs that form in rad24 mutants are ZIP1-independent and that Rad24 and Zip1 contribute to the same pathway for CO formation. Furthermore, the results indicate that the increase in NCO levels observed in the zip1 mutant depends on Rad24, suggesting that Rad24 functions upstream of Zip1 to channel intermediates to become interhomolog COs or NCOs rather than EC recombinants.

Finally, the rad24 zip1 double mutant shows a very similar defect in DSB repair as the rad24 single mutant. The double mutant also displayed similar hyper-resection compared to wild type and (the zip1) as the rad24 single (Figure 3, D and E). These data lead us to conclude that the Rad24 clamp loader, and therefore probably the 9-1-1 clamp, promotes ZIP1-dependent CO events.

**Discussion**

Rather than simply regulating cell cycle progression, DNA damage response (DDR) proteins play multiple roles in DNA metabolism during meiosis. These
roles include suppression of non-allelic homologous recombination (NAHR) and DSB formation, as well as promotion of IH bias, CO formation, and CO control. The 9-1-1 clamp loader/clamp and Mec1(ATR) kinase have distinct functions during meiotic prophase I. Although both the 911 clamp loader/clamp and Mec1(ATR) are required for suppression of NAHR, normal levels of meiotic CO formation require DDR clamp loader/clamp, but not Mec1. The DDR clamp loader/clamp promotes the functions of ZMM proteins during CO formation, likely via its direct interaction with Zip3 (SHINOHARA et al. 2015). Although previous studies analyzed the role of DDR proteins in CO formation, the roles of DDR protein in CO control had not been tested. Here, we carried out genetic analysis of a rad24 null mutant and various mec1 mutants. Taken together, the data suggest that Rad24 and Mec1 play distinct roles in regulating meiotic CO formation and distribution. One type of CO control, CO interference, which can be measured genetically or cytologically, consists of at least two distinct stages; establishment and maintenance (ZHANG et al. 2014a; ZHANG et al. 2014b). Previous studies showed the role of ZMM proteins in the maintenance of the
interference (ZHANG et al. 2014a; ZHANG et al. 2014b). In the \textit{zmm} mutants, the sites of COs appear to be designated and display the even spacing indicative of interference, but are not matured into CO products, but rather into NCOs. Given the similarity in defects in CO formation and control between ZMM and DDR mutants, we speculate that, like ZMM, Rad24 and Mec1 are both involved in CO maintenance, but their roles in maintenance are distinct.

We carried out genetic analysis of viable spores of several DDR mutants after tetrad dissection. This method relies on spore viability. The reliance on 4-viable spore tetrads in mutants that reduce spore viability could have biased the results. On the other hand, we tested three different alleles of the \textit{mec1} mutant, which show different spore viability from 48\% of \textit{mec1} null to 76\% for \textit{mec1}-\textit{kd}. Importantly, as shown above, all three \textit{mec1} mutants show the similar phenotype in terms of CO/NCO formation and CO interference. This argues against a strong impact of selecting for 4-viable spore tetrads on the recombination associated phenomena examined, at least in the case of the \textit{mec1} mutants. Furthermore, selection for 4-viable-spore tetrads is likely to
underestimate the impact of a recombination-defective mutant rather than exaggerate it.

The defect in CO interference in rad24 is reminiscent of zmm mutants. On the other hand, our results reveal new differences between rad24 and zmm. The rad24 mutant reduced CO frequencies to half of those in wild type in all intervals on both chromosome III and V, but did not reduce CO frequencies in several intervals on a long chromosome, chromosome VII. COs on all three chromosomes in the rad24 mutant do not show the interference. In contrast, a typical zmm mutant, zip3, reduced CO frequencies irrespective of chromosome size (CHEN et al. 2008; OKE et al. 2014). One possible explanation for wild-type levels of COs on chromosome VII in the rad24 mutant is that increases in ZMM-independent COs might compensate for loss of ZMM-dependent COs, but do so only on long chromosomes. A recent study showed that SC assembly down-regulates DSB formation during middle and late prophase I (THACKER et al. 2014). Given the rad24 mutant is defective in SC formation (GRUSHCOW et al. 1999), the mutant may display higher levels of DSB formation on longer
chromosomes than shorter chromosomes in late prophase I due to incomplete synapsis. If so, such late DSBs might contribute to ZMM-independent CO formation, since the mutant is deficient in ZMM-dependent CO formation. In this scenario, Rad24 might block ZMM-independent COs that otherwise arise from late DSBs on long chromosomes. Alternatively, long chromosomes may have a property that allows recruitment of ZMM independently of Rad24.

The rad24 and mec1 mutations confer different defects with respect to CO frequency and distribution. The simple explanation on the mec1 phenotypes in meiotic CO formation and control is that normal channeling of intermediates to the ZMM-dependent CO pathway, which is subject to interference, is lost in mec1 such that COs occur by a ZMM-independent, non-interfering, CO pathway. The Mec1 may activate the ZMM pathway and/or suppress a ZMM-independent pathway. The mec1 kinase dead mutant also shows CO phenotypes similar to those in the null mutant, suggesting the phosphorylation by Mec1 kinase is involved in regulation of COs. If Mec1 activates the ZMM-dependent pathway, such regulation is likely to occur after the assembly of ZMM complexes on
chromosomes, because the *mec1* mutant is proficient in ZMM focus formation (Shinohara *et al.* 2015).

An alternative model is based on the observation that Mec1(ADR) suppresses DSB formation through DSB trans-interference (Zhang *et al.* 2011; Garcia *et al.* 2015). In this model, the *mec1* mutants undergo more DSB formation than wild type. Indeed, our genetic analysis indicates that *mec1* mutants display increased NCO frequencies at most loci assayed, which is a result expected if more DSBs occur per meiosis in a given interval. If the additional DSBs are processed via a ZMM-independent CO pathway, which also forms NCO, that would account for the *mec1* defects described in this study. Even in this scenario, Mec1 seems to select the fate of the additional DSBs that are resolved by either ZMM-dependent or ZMM-independent mechanisms.

A recent study of whole-genome recombination analysis using hybrid yeast strains reached the same conclusion as we do here regarding the distinct regulatory roles of *rad24* and *mec1* in CO formation and control during meiosis (Crawford... and Neale *et al.* BioRxiv).
The *mec1* mutants we examined display chromosome-specific defects in CO frequency, which may reflect a mechanism that depends on chromosome size. The data are consistent with Mec1 enhancing the frequency of CO formation on small chromosomes, such as chromosome *III*, while it suppresses CO formation on long chromosomes, such as chromosome *VII*. Consistent with this, the *mec1* mutant does not affect CO frequencies on a medium length chromosome, chromosome *V*. The different effects of Mec1 kinase on COs seen on different intervals on different chromosomes suggest Mec1 senses some chromosome- or interval-specific property, with regulation based on chromosome size likely. In this context it should be pointed out that previous studies by Kaback and colleagues showed that both CO frequency and the strength of interference depends on chromosome size (*KABACK et al. 1992; KABACK et al. 1999*). These studies showed that long chromosomes display more robust interference than small chromosomes. Our results are consistent with the possibility that Mec1 is involved in size-dependent control to distribute COs among different chromosomes such that shorter chromosomes are assured
of undergoing the CO events required for their proper segregation. This type of control cannot be accounted for as a consequence of trans DSB interference, because DSB interference only acts over short distances on a given chromosome (GARCIA et al. 2015).

In closing we emphasize that, although our data demonstrate distinct roles for Rad24 and Mec1 in generating the normal distribution of CO events, genetic data of the type presented here cannot distinguish between interference mutants that fail to establish a non-random pattern of potential CO sites and interference mutants that fail to maintain the non-random pattern until CO products are eventually formed (ZHANG et al. 2014a). Cytological studies will be required to determine if RAD24 and/or MEC1 play a role in establishment verses maintenance of CO patterns.

ACKNOWLEDGEMENTS

We acknowledge Dr. N. Hunter for critical reading and are grateful for Dr. Matt Neale for sharing unpublished results prior to publication. We thank Ms. A.
Murakami and H. Wakabayashi for excellent technical assistance. This work was supported by JSPS KAKENHI Grant Number; 22125001, 22125002, 15H05973 and 16H04742 to A.S. M.S. was supported by the Japan Society for the Promotion of Science (JSPS) through the Funding Program for Next Generation World-Leading Researchers (NEXT Program), and D.K.B. was supported by US NIH grant GM50936.

**Author's contribution**

M.S., D.K.B., and A.S. designed experiments. M.S. performed all experiments and analyzed the data. M.S., D.K.B, and A.S. prepared the manuscript.
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Figure legends

Figure 1. The \textit{rad24} and \textit{mec1} mutants are differentially defective in crossover frequency.

(A) Schematic representation of markers on Chromosome V.

(B) Genetic maps of three different chromosomes in wild type (blue bars), the \textit{rad24} (magenta bars), \textit{mec1 sml1-X} mutants (green bars) and \textit{mec1 sml1-X} mutants (light green bars). See Table 1, 3 and 4 for more details. Map distances were calculated using the Perkins equation.

(C) Genetic maps of different intervals on three different chromosomes in wild type (blue bars), the \textit{rad24} (magenta bars), \textit{mec1 sml1-X} mutants (green bars) and \textit{mec1 sml1-X} mutants (light green bars). See Table 1, 3 and 4 for more details. Error bars indicate SE.

Figure 2. The \textit{rad24} and \textit{mec1} mutants are defective in crossover interference.
(A) The $\text{NPD}_{\text{observed}}/\text{NPD}_{\text{expected}}$ ratios for eight intervals in wild type (blue), the
$\text{rad2}4$ (red) a $\text{mec}1 \text{ sml}1-\text{X}$ mutants (green) and $\text{mec}1-\text{kd} \text{ sml}1-\text{X}$ (light
green) were calculated as described in Methods and shown on chromosome
$V$, $\text{III}$ and $\text{VII}$ (B). Also see Table 1, 3 and 4.

(B) Coefficient of coincidence (CoC) of CO frequencies of two adjacent intervals
are calculated and shown on chromosome $V$, $\text{III}$ and $\text{VII}$. Also see Table S2,
S3 and S4.

**Figure 3. RAD24 functions upstream of ZIP1 in meiotic recombination.**

(A) Schematic representation of $\text{HIS4-LEU2}$ and $\text{leu}2::\text{hisG}$ loci. HD assay, left;
DSB assay, right. In HD assay, DSB bands contain single-stranded DNA
tails (shown by dotted lines; DSB1, DSB2), which confers resistance to
digestion by restriction enzyme.

(B) Heteroduplex (HD) formation in wild type and the various mutants was
analyzed by Southern blotting. Genomic DNAs were digested with $\text{BamHI}$,
$\text{MluI}$ and $\text{XhoI}$. 

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(C) Quantification of NCO-, CO- and EC-HDs is shown in each graph. Wild type, blue (n=3); rad24, red (n=3); zip1, green (n=1); rad24 zip1, purple (n=3).

Note: interpretation of HD band levels assumes that the mutations examined do not alter the efficiency of mismatch repair of the MluI/BamHI HDs.

(D) DSB blots in various mutants. DSBs were analyzed by Southern blotting.

Genomic DNAs were digested with PstI. Variable resection of DNA ends is likely to account for the low levels of DSB-specific bands in rad24 zip1.

(E) Quantification of kinetics of DSB repair shown in (D). Wild type, blue circles: zip1, green triangles; rad24, red circles; rad24 zip1, purple triangles.
Table 1. Genetic analysis of the meiotic recombination on chromosome V in *rad24* and *mec1* mutants

| Intervals          | PD  | TT  | NPDo | Total | cM (S.E.) | NPD ratio | P-value for difference from WT | P-value for difference from one |
|--------------------|-----|-----|------|-------|-----------|-----------|--------------------------------|--------------------------------|
| Wild type          |     |     |      |       |           |           |                                 |                                 |
| CAN1-URA3          | 313 | 744 | 29   | 1086  | 42.2±1.5  | 0.24      | -                              | 6.1 X 10^{-17}                  |
| URA3-HOM3          | 343 | 696 | 43   | 1082  | 44.1±1.7  | 0.32      | -                              | 7.4 X 10^{-15}                  |
| HOM3-TRP2          | 824 | 240 | 3    | 1067  | 12.1±0.8  | 0.37      | -                              | 0.077                          |
| rad24              |     |     |      |       |           |           |                                 |                                 |
| CAN1-URA3          | 804 | 358 | 22   | 1184  | 20.7±1.3  | 1.27      | 1.1 X 10^{-156}               | 0.23                           |
| URA3-HOM3          | 737 | 420 | 22   | 1179  | 23.4±1.3  | 0.87      | 3.3 X 10^{-90}                | 0.55                           |
| HOM3-TRP2          | 101 | 146 | 2    | 1167  | 6.8±0.6   | 0.8       | 4.4 X 10^{-22}                | 1                              |
| mec1 sml1-X        |     |     |      |       |           |           |                                 |                                 |
| CAN1-URA3          | 496 | 524 | 48   | 1068  | 38.1±1.9  | 0.92      | 5.4 X 10^{-19}                | 0.58                           |
| URA3-HOM3          | 418 | 562 | 69   | 1049  | 46.5±2.2  | 1.04      | 1.4 X 10^{-12}                | 0.71                           |
| HOM3-TRP2          | 719 | 287 | 17   | 1023  | 19.3±1.0  | 1.35      | 3.1 X 10^{-8}                 | 0.27                           |

NPDo is a number of NPD observed in crosses.

*a* Map distances and NPD ratio with S.E. were calculated using the Stahl Lab online tool ([http://www.molbio.uoregon.edu/~fstahl](http://www.molbio.uoregon.edu/~fstahl)). NPDe is a number of NPD expected from Papazian equation.

*b* Differences between the wild type and the *rad24* mutant were calculated using chi-square independent test.

*c* Differences between NPDo and NPDe were calculated using chi-square independent test.
### Table 2. Non-Mendelian segregation in rad24 and mec1 mutants

|                     | Percent of non-Mendelian segregation |
|---------------------|--------------------------------------|
|                     | CAN1      | URA3 | HOM3   | TRP2   |
| Wild type           | 1.2       | 0.3  | 1.5    | 1.8    |
| *rad24*             | 0.67      | 0.08 | 1.17   | 0.11   |
|                     | (0.56)    | (0.27)| (0.78) | (0.06) |
| *mec1 sml1-X*       | 1.2       | 0.64 | 2.9    | 3.2    |
|                     | (1)       | (2.1)| (1.9)  | (1.8)  |

Frequencies of tetrad with 4+:0−, 3+:1−, 1+:3− and 0+:4− segregation for each marker were calculated. Relative ratios of the frequencies in the mutant to wild type are shown in parenthesis.
Table 3. Genetic analysis of meiotic recombination on chromosome III in rad24 and mec1 mutants

| Intervals                  | PD  | TT  | NPD o | Total  | NPDc | cM (S.E.)\(^a\) | NPD ratio\(^b\) | P-value for difference from WT\(^b\) | P-value for difference from NPDc\(^c\) |
|----------------------------|-----|-----|-------|--------|------|-----------------|-----------------|-------------------------------------|--------------------------------------|
| Wild type                  |     |     |       |        |      |                 |                 |                                     |                                      |
| HML-URA3                  | 1031| 286 | 2     | 1301   | 9.30 | 11.4±0.65       | 0.0071          | -                                   | 0.017                                |
| URA3-LEU2                 | 908 | 312 | 7     | 1227   | 12.1 | 14.9±0.9        | 0.0099          | -                                   | 0.14                                 |
| LEU2-HIS4                 | 1173| 55  | 0     | 1228   | 0.31 | 2.2±0.3         | NA              | -                                   | 0.58                                 |
| HIS4-MAT                  | 533 | 712 | 49    | 1294   | 45.8 | 39.1±1.7        | 0.0069          |                                     | 0.64                                 |
| rad24                     |     |     |       |        |      |                 |                 |                                     |                                      |
| HML-URA3                  | 1191| 104 | 1     | 1296   | 1.1  | 4.2±0.44        | 0.91            | 9.0 X 10\(^{-75}\)                     | 0.92                                 |
| URA3-LEU2                 | 1069| 125 | 2     | 1196   | 1.1  | 5.7±0.57        | 1.13            | 1.9 X 10\(^{69}\)                      | 0.88                                 |
| LEU2-HIS4                 | 1182| 150 | 0     | 1197   | 0.02 | 0.6±0.43        | NA              | 8.4 X 10\(^{15}\)                     | 0.89                                 |
| HIS4-MAT                  | 734 | 500 | 36    | 1270   | 34.9 | 28.2±1.6        | 1.03            | 3.2 X 10\(^{33}\)                     | 0.85                                 |
| mec1                      |     |     |       |        |      |                 |                 |                                     |                                      |
| sml1-X                    |     |     |       |        |      |                 |                 |                                     |                                      |
| HML-URA3                  | 1095| 90  | 2     | 1187   | 0.90 | 4.3±0.52        | 2.22            | 3.2 X 10\(^{94}\)                     | 0.25                                 |
| URA3-LEU2                 | 896 | 190 | 5     | 1091   | 4.70 | 10.1±0.84       | 1.06            | 6.0 X 10\(^{18}\)                     | 0.89                                 |
| LEU2-HIS4                 | 1084| 22  | 0     | 1106   | 0    | 1±0.21          | NA              | 4.8 X 10\(^{14}\)                     | NA                                   |
| HIS4-MAT                  | 551 | 559 | 52    | 1162   | 53.8 | 37.5±2.0        | 0.97            | 5.5 X 10\(^{10}\)                     | 0.81                                 |
| mec1-kd                    |     |     |       |        |      |                 |                 |                                     |                                      |
| sml1-X                    |     |     |       |        |      |                 |                 |                                     |                                      |
| HML-URA3                  | 1077| 177 | 2     | 1186   | 3.68 | 8.0±0.6         | 0.54            | 9.9 X 10\(^{16}\)                     | 0.38                                 |
| URA3-LEU2                 | 826 | 234 | 6     | 1066   | 7.59 | 12.7±0.94       | 0.79            | 3.6 X 10\(^{8}\)                      | 0.56                                 |
| LEU2-HIS4                 | 1043| 40  | 0     | 1083   | 0    | 1.8±0.29        | NA              | 3.0 X 10\(^{6}\)                      | NA                                   |

\(^a\) Values based on 1000 bootstrap replicates

\(^b\) Mann-Whitney U-test
|        | HIS4-MAT | 639 | 78 | 1158 | 80.2 | 47.8±2.3 | 0.97 | 4.8 X 10^9 | 0.81 |
|--------|----------|-----|----|------|------|----------|------|-------------|------|
|        |          |     |    |      |      |          |      |             |      |

NPD<sub>o</sub> is a number of NPD observed in crosses.

<sup>a</sup>Map distances and NPD ratio with S.E. were calculated using the Stahl Lab online tool ([http://www.molbio.uoregon.edu/~fstahl](http://www.molbio.uoregon.edu/~fstahl)). NPD<sub>e</sub> is a number of NPD expected from Papazian equation. The ratio of mutant to wild type map distance is shown in parenthesis.

<sup>b</sup>Differences between the wild type and the rad24 mutant were calculated using chi-square independent test.

<sup>c</sup>Differences between NPD<sub>o</sub> and NPD<sub>e</sub> were calculated using chi-square independent test.
| Intervals                | PD   | TT   | NPD0 | Total | NPDc (S.E.)  | NPD ratio (S.E.) | P-value for difference from WT | P-value for difference from NPDc |
|--------------------------|------|------|------|-------|-------------|-----------------|-------------------------------|---------------------------------|
| **Wild type**            |      |      |      |       |             |                 |                               |                                 |
| CUP2-MET13               | 531  | 630  | 17   | 1178  | 31.1±1.3    | 0.063           | -                             | 3.4 X 10^-11                    |
| MET13-CYH2               | 964  | 273  | 0    | 1237  | 11.0±0.59   | 0.007           | -                             | -                               |
| CYH2-TRP5                | 483  | 756  | 45   | 1284  | 40±1.7      | 0.086           | -                             | -                               |
| TRP5-ADE6                | 387  | 798  | 91   | 1276  | 52.7±2.3    | 0.11            | -                             | 3.7 X 10^-10                    |
| CUP2-MET13               | 645  | 482  | 42   | 1169  | 13.4±1.8    | 1.17            | 3.4 X 10^-18                  | 0.32                            |
| **rad24**                |      |      |      |       |             |                 |                               |                                 |
| MET13-CYH2               | 986  | 236  | 3    | 1225  | 10.4±0.7    | 0.46            | 9.6 X 10^-3                  | 1.6                             |
| CYH2-TRP5                | 499  | 682  | 81   | 1262  | 46.3±2.2    | 0.99            | 4.7 X 10^-6                  | 0.92                            |
| TRP5-ADE6                | 453  | 693  | 95   | 1241  | 50.9±2.4    | 1.07            | 1.1 X 10^-12                 | 0.55                            |
| **mec1**                 |      |      |      |       |             |                 |                               |                                 |
| CUP2-MET13               | 414  | 576  | 43   | 1033  | 40.4±2.0    | 0.58            | 2.0 X 10^-11                  | 3.1 X 10^-4                    |
| MET13-CYH2               | 830  | 279  | 6    | 1115  | 14.1±0.92   | 0.57            | 9.3 X 10^-7                  | 0.16                            |
| CYH2-TRP5                | 351  | 695  | 95   | 1141  | 55.4±2.6    | 0.85±           | 2.2 X 10^-18                 | 0.13                            |
| TRP5-ADE6                | 326  | 674  | 108  | 1108  | 59.7±2.8    | 1.0             | 6.4 X 10^-17                 | 0.92                            |
| **mec1-kd**              |      |      |      |       |             |                 |                               |                                 |
| CUP2-MET13               | 389  | 615  | 45   | 1049  | 42.2±2.0    | 0.51            | 7.6 X 10^-16                  | 3.1 X 10^-6                    |
| MET13-CYH2               | 805  | 305  | 5    | 1115  | 15±0.9     | 0.39            | 2.3 X 10^-9                  | 0.026                           |
| CYH2-TRP5                | 300  | 773  | 97   | 1170  | 57.9±2.5   | 0.56            | 4.2 X 10^-31                 | 7.6 X 10^-9                    |
| TRP5-ADE6                | 299  | 708  | 112  | 1119  | 61.7±2.8   | 0.87            | 2.5 X 10^-18                 | 0.13                            |
NPDo is a number of NPD observed in crosses.

*a* Map distances and NPD ratio with S.E. were calculated using the Stahl Lab online tool (http://www.molbio.uoregon.edu/~fstahl). NPDe is a number of NPD expected from Papazian equation.

*b* Differences between the wild type and the rad24 mutant were calculated using chi-square independent test.

*c* Differences between NPDo and NPDe were calculated using chi-square independent test.
Table 5. Non-Mendelian segregation in rad24 and mec1 mutants

|                  | Percent of non-Mendelian segregation | Percent of non-Mendelian segregation |
|------------------|--------------------------------------|--------------------------------------|
|                  | On Chromosome III                     | On Chromosome VII                    |
|                  | HML  | URA3 | LEU2 | HIS4 | MAT | CUP2 | MET13 | CYH2 | TRP5 | ADE6 |
| Wild type        | 0.46 | 0.99 | 5.85 | 1.06 | 0.84| 5.92 | 5.39 | 1.21 | 1.51 | 1.82 |
| rad24            | 0.45 | 1.36 | 8.11 | 2.12 | 1.59| 5.53 | 6.14 | 1.06 | 3.26 | 2.80 |
| (0.98)           | (1.37)| (1.39)| (2.00)| (1.89)| (0.93)| (1.14)| (0.88)| (2.16)| (1.54)| |
| mec1             | 0.16 | 2.06 | 8.24 | 1.81 | 2.39| 9.15 | 6.51 | 1.98 | 4.29 | 4.86 |
| sml1-X           | (0.35)| (2.08)| (1.41)| (1.71)| (2.84)| (1.54)| (1.21)| (1.64)| (2.84)| (2.67)| |
| mec1-kd          | 0.08 | 3.18 | 10.4 | 2.20 | 3.59| 7.17 | 7.91 | 1.55 | 3.18 | 5.71 |
| sml1-X           | (0.17)| (3.21)| (1.78)| (2.08)| (4.27)| (1.21)| (1.46)| (1.28)| (2.11)| (3.14)| |

Frequencies of tetrads with $4^+\cdot 0^-$, $3^+\cdot 1^-$, $1^+\cdot 3^-$ and $0^+\cdot 4^-$ segregation for each marker were calculated. Ratios of the frequencies in the mutant to wild type are shown in parenthesis.
Figure 1. Miki Shinohara

A

Ch. V

CAN1-URA3-HOM3-TRP2

Ch. III

HML-URA3-LEU2-HIS4-URA3-LEU2

Ch. VII

CUP2-MET13-CYH2-TRP5

B

Crossover (total)

Ch. V

Ch. III

Ch. VII

C

Crossover

D

Crossover

Ch. III

Ch. VII
Figure 2. Miki Shinohara

A

Intereference

NPD ratios

Ch. V

Ch. III

Ch. VII

Wild type
rad24
mec1
mec1-kd

B

CoC

Wild type
rad24
mec1
mec1-kd

Ch. V

Ch. III

Ch. VII
Figure 3. Miki Shinohara

A

B

C

D

E

Figure 3. Miki Shinohara
| Strain | Description |
|--------|-------------|
| NKY1551 | MATα, ho::LYS2/*, lys2/*, ura3/*, leu2::hisG/*, his4X-LEU2(BamHI)-URA3/his4B-LEU2(MluI), arg4-nsp/arg4-bgl |
| MSY717 | NKY1551 with rad24::LEU2 |
| MSY2820 | NKY1551 with zip1::LEU2 |
| MSY2122 | NKY1551 with rad24::LEU2, zip1::LEU2 |
| MSY620 | MATα, ho::LYS2, lys2, leu2::hisG, ura3, hom3-10, trp2 |
| S2921 | MATα, ho::LYS2, lys2, leu2::hisG, can1-R |
| MSY1172 | MATα, ho::LYS2, lys2, ura3, hom3-10, trp2 |
| MSY1174 | MATα, ho::LYS2, lys2, can1-R |
| MSY654 | S2921 with rad24::LEU2 |
| MSY655 | MSY620 with rad24::LEU2 |
| MSY1222 | S2921 with mec1::LEU2, sml1-X |
| MSY1275 | MSY620 with mec1::LEU2, sml1-X |
| MSY4245 | MATα, ho::LYS2, lys2, hml::KanMX6, HIS4-LEU2-URA3, cyh2-R, arg4-bgl |
| MSY4303 | MATα, ho::LYS2, lys2, his4B-leu2-E, cup2-B, met13-B, trp5-S, ade6-B, arg4-bgl |
| MSY4360 | MSY4245 with mec1-1 sml1-X |
| MSY4358 | MSY4360 with mec1-1 sml1-X |
| MSY4417 | MSY4245 with sml1-X |
| MSY4419 | MSY4245 with mec1-kd::Xbal, sml1-X |
| MSY4429 | MSY4360 with sml1-X |
| MSY4435 | MSY4360 with mec1-kd::Xbal, sml1-X |
| MSY5247 | MSY4245 with rad24::HygMX6 |
| MSY5330 | MSY4360 with rad24::HygMX6 |
|                   | Double crossovers in    | Double crossovers in    |
|-------------------|-------------------------|-------------------------|
|                   | CAN1-URA3-HOM3          | URA3-HOM3-TRP2          |
| Observed          | Expected                 | Ratio                   | Observed          | Expected                 | Ratio                   |
| (Total)           | (P-value)                |                          | (Total)           | (P-value)                |                          |
| WT                | 0.43                    | 0.48                    | 0.90              | 0.15                    | 0.16                    | 0.94                    |
| (1067)            | (0.018)                 |                          | (1067)            | (0.41)                  |                          |
| rad24             | 0.16                    | 0.12                    | 1.33              | 0.069                   | 0.043                   | 1.60                    |
| (1167)            | (8.0X10^-5)             |                          | (1167)            | (1.8X10^-5)             |                          |
| mec1              | 0.28                    | 0.20                    | 1.4               | 0.15                    | 0.10                    | 1.5                     |
| (1170)            | (9.4X10^-10)            |                          | (1170)            | (6.4X10^-8)             |                          |

*aThe number of tetrads with two adjacent intervals, both of which show crossovers (either tetrate or non-parental ditype), was counted. In parenthesis, a total number of tetrads checked is shown.

*bExpected is the products of the numbers given for two adjacent intervals with crossovers from the number of a crossover in single intervals.

*cP-value reflects the likelihood for the differences between the expected and observed frequencies is attributable to a chance as determined by $\chi^2$ test.
### Table S3. Coincidence of crossovers on chromosome III

|                      | Double crossovers in HML-URA3-LEU2 | Double crossovers in URA3-LEU2-HIS4 | Double crossovers in LEU2-HIS4-MAT |
|----------------------|-------------------------------------|-------------------------------------|-------------------------------------|
|                      | Observed<sup>a</sup>  | Expected<sup>b</sup> | Ratio  | Observed<sup>a</sup>  | Expected<sup>b</sup> | Ratio  | Observed<sup>a</sup>  | Expected<sup>b</sup> | Ratio  |
|                      | (Total)         | (P-value)           |        | (Total)         | (P-value)           |        | (Total)         | (P-value)           |        |
| WT                   | 0.033           | 0.061               | 0.53   | 0.0091           | 0.013               | 0.7    | 0.021           | 0.03               | 0.7    |
|                      | (1104)          | (1.7X10<sup>-4</sup>) |        | (1104)          | (0.26)               |        | (1212)          | (0.084)            |        |
| rad24                | 0.0078          | 0.0078              | 1      | 0                | 0.0012              | NA     | 0.0078          | 0.0050             | 1.6    |
|                      | (1160)          | (1)                 |        | (1160)          | (1160)              |        | (1319)          | (0.18)             |        |
| mecl-1               | 0.011           | 0.014               | 0.79   | 0.0038           | 0.0034              | 1.1    | 0.0076          | 0.01               | 0.76   |
|                      | (1061)          | (0.41)              |        | (1061)          | (0.82)              |        | (1213)          | (0.42)             |        |
| mecl-kd              | 0.039           | 0.034               | 1.1    | 0.012            | 0.0085              | 1.4    | 0.015           | 0.022              | 0.7    |
|                      | (1033)          | (0.38)              |        | (1033)          | (0.22)              |        | (1226)          | (0.13)             |        |

<sup>a</sup>The number of tetrads with two adjacent intervals, both of which show crossovers (either tetratype or non-parental ditype), was counted. In parenthesis, a total number of tetrads checked is shown.

<sup>b</sup>Expected is the products of the numbers given for two adjacent intervals with crossovers from the number of a crossover in single intervals.

<sup>c</sup>P-value reflects the likelihood for the differences between the expected and observed frequencies is attributable to a chance as determined by \( \chi^2 \) test.
### Table S4. Coincidence of crossovers on chromosome VII

|                    | Double crossovers in CUP2-MET13-CYH2 | Double crossovers in MET13-CYH2-TRP5 | Double crossovers in CYH2-TRP5-ADE6 |
|--------------------|--------------------------------------|--------------------------------------|--------------------------------------|
| Observed\(a\)     | Expected\(b\)                        | Ratio                                | Observed\(a\)                        | Expected\(b\)                        | Ratio                                | Observed\(a\)                        | Expected\(b\)                        | Ratio                                |
| (Total)            | (P-value)                             |                                       | (Total)                              | (P-value)                             |                                       | (Total)                              | (P-value)                             |                                       |
| WT                 | 0.070                                | 0.12                                 | 0.13                                 | 0.15                                 | 0.86                                 | 0.45                                 | 0.47                                 | 0.95                                 |
|                    | (1054)                               | (3.7X10\(^{-5}\))                    | (1054)                               | (0.094)                              | (1054)                               | (0.34)                               |
| rad24              | 0.099                                | 0.087                                | 0.12                                 | 0.11                                 | 1.10                                 | 0.41                                 | 0.38                                 | 1.1                                  |
|                    | (1092)                               | (0.17)                               | (1092)                               | (0.32)                               | (1092)                               | (0.11)                               |
| mec1-1             | 0.13                                 | 0.14                                 | 0.16                                 | 0.16                                 | 1                                    | 0.47                                 | 0.48                                 | 0.98                                 |
|                    | (932)                                | (0.41)                               | (932)                                | (1)                                  | (932)                                | (0.66)                               |
| mec1-kd            | 0.15                                 | 0.17                                 | 0.18                                 | 0.20                                 | 0.90                                 | 0.53                                 | 0.53                                 | 1                                    |
|                    | (955)                                | (0.13)                               | (955)                                | (0.17)                               | (955)                                | (1)                                  |

\(a\) The number of tetrads with two adjacent intervals, both of which show crossovers (either tetratype or non-parental ditype), was counted. In parenthesis, a total number of tetrads checked is shown.

\(b\) Expected is the products of the numbers given for two adjacent intervals with crossovers from the number of a crossover in single intervals.

\(c\) P-value reflects the likelihood for the differences between the expected and observed frequencies is attributable to a chance as determined by \(\chi^2\) test.
Supplementary Figure legends:

Figure S1. Distribution of viable spores in the rad24 and mec1 mutants.
A. Distribution of viable spores per tetrad. Numbers of viable spores per tetrad were calculated for wild type (blue bars), the rad24 (magenta bars) and mec1 sml1-X mutants (green bars).
B. Distribution of viable spores per tetrad. Numbers of viable spores per tetrad were calculated for wild type (blue bars), the rad24 (magenta bars), mec1-1 sml1-X mutants (green bars) and mec1-kd sml1-X mutants (light green bars).
Supplementary Figure 1. Miki Shinohara

A

Distribution of viable spores

Percent of tetrad

Wild type (N=1196)
rad24 (N=8060)
mec1 (N=5570)

B

Distribution of viable spores

Percent of tetrad

Wild type
rad24
mec1-1
mec1kd