The Rad1-Rad10 nuclease promotes chromosome translocations between dispersed repeats

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Homologous recombination is an important repair mechanism to eliminate double-strand breaks (DSBs) and to bypass lesions that result in formation of single-stranded DNA gaps during DNA replication1. Homologous recombination can result in the exchange of flanking markers to produce crossovers between chromosomes. Although crossovers are essential during meiosis, they can have adverse consequences in somatic (mitotic) cells. A crossover between nonsister chromatids in G2 cells results in loss of heterozygosity (LOH) from sequences in somatic cells. A crossover between dispersed repeats are essential during meiosis, they can have adverse consequences to produce crossovers between chromosomes. Although crossovers were not recovered from the mus81Δ rad1Δ yen1Δ triple mutant, indicating that all three nucleases participate in processing recombination intermediates that form between dispersed repeats. We suggest a new mechanism for crossovers that involves Rad1-Rad10 clipping and resolution of a single Holliday junction–containing intermediate by Mus81-Mms4 or Yen1 cleavage or by replication. Consistent with the model, we show accumulation of Rad1-dependent joint molecules in the mus81Δ yen1Δ mutant.

Holliday junctions can be formed during homology-dependent repair of DNA double-strand breaks, and their resolution is essential for chromosome segregation and generation of crossover products. The Mus81-Mms4 and Yen1 nucleases are required for mitotic crossovers between chromosome homologs in Saccharomyces cerevisiae; however, crossovers between dispersed repeats are still detected in their absence. Here we show that the Rad1-Rad10 nuclease promotes formation of crossover and noncrossover recombinants between ectopic sequences. Crossover products were not recovered from the mus81Δ rad1Δ yen1Δ mutant shows normal meiotic recombination but for mitotic crossovers between chromosome homologs in S. cerevisiae, with Yen1 serving a backup function, and it is essential for meiotic crossovers in Schizosaccharomyces pombe19–24. Most meiotic crossovers in S. cerevisiae result from biased resolution of dHJ intermediates by Mlh1-Mlh3-Exo1 and meiosis-specific ZMM factors25–26. However, Mus81-Mms4 is primarily responsible for the meiotic crossovers generated by the interference-independent pathway, which might be considered analogous to mitotic crossovers and the S. pombe meiotic crossover pathway25,26. Mitotic-crossover products are still detected between dispersed repeats in the mus81Δ yen1Δ mutant, suggesting that an additional activity or alternative mode of processing recombination intermediates operates in this context27. The Rad1-Rad10 (XPF-ERCC1 in mammals) nuclease, which is essential for nucleotide excision repair28, is suggested to resolve meiotic recombination intermediates in some organisms29. The S. cerevisiae rad1Δ mutant shows normal meiotic recombination but

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is defective for mitotic recombination between substrates that require removal of heterologous flaps. In addition, rad1∆ and mouse Erc1 cells are defective for integration of linear DNA fragments to replace chromosomal sequences during gene targeting, leading to the proposal that Rad1-Rad10 (XPF-ERCC1) cleaves at the boundary between homologous and heterologous sequences, represented by the selected marker, during this process.

Here we sought to test the hypothesis that Rad1-Rad10 cleaves recombination intermediates formed at the heterology barrier, resulting in the generation of crossover products between dispersed repeats. Using two different assays to measure recombination between homology-limited substrates, we show that Rad1 is required for normal levels of crossovers and that in the absence of Mus81, Rad1 and Yen1 crossovers are eliminated. In addition, we provide physical evidence that Rad1-Rad10 cleaves a recombination intermediate to generate a substrate that is dependent on Mus81-Mms4 or Yen1 for resolution.

RESULTS

Rad1 is required for plasmid integration

To test the role of Rad1 in recombination between homology-limited substrates, we first analyzed integration of a linearized plasmid at the homologous chromosomal locus during transformation of yeast cells. An ARS-containing (autonomously replicating) plasmid containing URA3 and MET17 genes was digested with two restriction enzymes to create a 238-base-pair (bp) double-strand DNA gap within the MET17 gene, which has a 2.6-kilobase (kb) homology to the chromosomal locus (Fig. 1a). Homology-dependent repair of the plasmid yields noncrossover (episomal) or crossover (integrated) products with an unstable or stable Ura+ phenotype, respectively. The overall frequency of gap repair was reduced in the rad1∆ mutant as compared with wild type (P = 0.008), and it decreased further in the mus81∆ rad1∆ (P = 0.04) and mus81∆ rad1∆ yen1∆ (P = 0.0008) mutants as compared with the rad1∆ single mutant (Fig. 1b). A previous study identified a role for Rad1-Rad10 in removal of heterologous sequences at the DSB ends during strand invasion; however, the ends of the plasmid are homologous to the chromosomal donor in this assay, indicating that the rad1∆ defect is at a later step of repair.

Independent Ura+ transformants were scored for mitotic stability to determine the frequency of gap repair with or without plasmid integration (Fig. 1b). In the wild type, 44% of the Ura+ transformants resulted from integration of the plasmid at the met17 locus. No crossover defect was found for the mus81∆ or yen1∆ single mutants, but crossovers were reduced in the rad1∆ mutant (P = 0.0018), consistent with previous studies. Unexpectedly, 75% of the Ura+ transformants recovered from the mus81∆ yen1∆ double mutant were due to plasmid integration, which resulted in a significant decrease in the noncrossover class (P = 0.0001). This contrasts with our previous study using chromosome homologs that demonstrated increased noncrossovers and decreased crossovers in the mus81∆ yen1∆ double mutant. The mus81∆ rad1∆ yen1∆ mutant showed a lower frequency of integration than did the rad1∆ single mutant (P = 0.008), indicating that Mus81-Mms4 and Yen1 contribute to plasmid integration in the absence of Rad1 and that the increased plasmid integration in the mus81∆ yen1∆ mutant is due to Rad1 activity (P = 0.0015). Southern blot analysis confirmed that the events scored genetically as crossovers from the mus81∆ yen1∆ and mus81∆ rad1∆ yen1∆ mutants were due to plasmid integration at the met17 locus (Supplementary Fig. 1). In addition, the frequency of noncrossover events was reduced in the triple mutant as compared with the rad1∆ single mutant (P = 0.016) but was not different from that in the mus81∆ yen1∆ double mutant. As discussed below, the decrease in formation of noncrossover products observed for the mus81∆ yen1∆ mutant suggests that resolution of recombination intermediates contributes to the noncrossover class.

Crossovers between repeats require Mus81, Rad1 and Yen1

To assess the roles of Rad1, Mus81 and Yen1 in DSB-induced recombination between dispersed chromosomal repeats, we used an assay that allows for recovery of both noncrossover and crossover (translocation) products. A previous study reported that DSB-induced translocations were significantly reduced in the rad1∆ mutant, but noncrossovers could not be detected by the system used. The haploid strains have a 39-bp HO-endonuclease cut site inserted within the native URA3 locus on chromosome V and a 5.6-kb ura3 fragment integrated at the LYS2 locus on chr. II (Fig. 2a). The donor ura3 allele includes a 39-bp insertion of the HOcs-inc (noncleavable) site, with a BamHI restriction site 6 bp from the noncleavable HO recognition sequence, to monitor noncrossover repair. Because the donor allele shares extensive homology to the cut locus (a 3-bp substitution to create the BamHI site and a 1-bp substitution on the other side of the break, owing to the inc mutation), there should be no requirement for Rad1-Rad10 flap cleavage during strand invasion.

Figure 1 Rad1 is required for plasmid integration. (a) Noncrossover (NCO) repair of the double-strand DNA gap within the MET17 gene yields an episomal plasmid, whereas repair associated with a crossover (CO) integrates the plasmid. (b) Gap-repair frequencies. WT, wild type. Error bars show s.e.m., and significance was determined by unpaired t test (WT, n = 13; mus81∆ rad1∆ yen1∆ mutant, n = 5; mus81∆ yen1∆, mus81∆ rad1∆, mus81∆ yen1∆ and rad1∆ yen1∆ mutants, n = 4; rad1∆ mutant, n = 3). For simplicity, the null alleles are written as mus81∆, rad1∆ and yen1∆ in the figure. The frequency of gap repair with or without integration was determined for 108 independent transformants from each trial.
After induction of HO, regulated by the galactose-inducible GAL1 promoter, the DSB is repaired by gene conversion transferring the HOcs-inc allele and BamHI site to the recipient locus. As an overall evaluation of repair, the plating efficiency of each strain on galactose-containing medium (HO constitutively expressed) was compared with the plating efficiency on medium containing glucose (HO off) (Fig. 2a). The rad1Δ mutant showed reduced plating efficiency on galactose-containing medium compared with the wild type (P = 0.0075), and the plating efficiencies of the mus81Δ rad1Δ and mus81Δ rad1Δ yen1Δ mutants were lower than for the rad1Δ single mutant (P = 0.015 and 0.0004, respectively). The yen1Δ and rad1Δ yen1Δ strains were not different from wild type and rad1Δ and were not analyzed further (Supplementary Fig. 2b).

To determine the fraction of repaired products with an associated crossover, HO was induced in liquid cultures, and DNA was isolated at different times after HO induction for ApaI and PvuII restriction digestion followed by Southern blot hybridization to detect fragments diagnostic of crossover products (Fig. 2a,c). In the wild type, crossover products accumulated to 8.4% of the DNA products 24 h after HO induction. The mus81Δ single mutant showed similar levels of crossover bands as the wild-type strain, and crossovers were reduced by a factor of two in the mus81Δ yen1Δ double mutant, as reported previously. The rad1Δ single mutant showed a small decrease in the level of crossover bands compared with the wild type (P = 0.011), and crossover products were reduced further in the mus81Δ rad1Δ and mus81Δ rad1Δ yen1Δ mutants as compared with the rad1Δ mutant (P < 0.001 for both mutants). These data suggest that Rad1-Rad10 and Mus81-Mms4 have partially redundant functions or cooperate to form crossovers between dispersed repeats.

Faint bands on Southern blots corresponding to the sizes of crossover products were detected in mus81Δ rad1Δ yen1Δ cells (Fig. 2). To determine whether these were bona fide reciprocal exchange products, wild-type, mus81Δ rad1Δ, mus81Δ yen1Δ and mus81Δ rad1Δ yen1Δ cells were plated on glucose-containing medium following an 8-h liquid induction of HO, and DNA isolated from pools of five independent colonies was analyzed by restriction digestion and Southern blot hybridization to detect crossovers. For pools with crossover fragments, individual clones were assayed to verify noncrossover or reciprocal crossover products. Eighteen of 324 colonies analyzed from the wild type, corrected for the 92% that were recombinants (transfer of the BamHI site to the cut locus), showed crossover bands (Fig. 3a,b). To ensure that the crossover DNA fragments represent chromosome translocations, one noncrossover and three independently derived crossover recombinants from the wild type were subjected to pulsed-field gel electrophoresis to separate intact chromosomes, followed by Southern blot hybridization using probes specific to chr. II or chr. V. The 587-kb chr. II-V and 803-kb chr. V-II reciprocal translocations, one noncrossover and three independently derived crossover recombinants from the wild type were subjected to pulsed-field gel electrophoresis to separate intact chromosomes, followed by Southern blot hybridization using probes specific to chr. II or chr. V. The 587-kb chr. II-V and 803-kb chr. V-II reciprocal translocations, one noncrossover and three independently derived crossover recombinants from the wild type were subjected to pulsed-field gel electrophoresis to separate intact chromosomes, followed by Southern blot hybridization using probes specific to chr. II or chr. V. The 587-kb chr. II-V and 803-kb chr. V-II reciprocal translocations were found in the three crossover clones (Fig. 3c).

No crossover products were found among 496 recombinant survivors of the mus81Δ rad1Δ yen1Δ triple mutant (P = 0.0001) (Fig. 3a,b). The faint bands detected in populations could be due to break-induced replication (BIR) resulting in nonreciprocal LOH, a lethal event in haploid cells. Analysis of 263 colonies of the mus81Δ rad1Δ mutant revealed three crossover clones, indicating a low level...
of Yen1-dependent cleavage to generate crossovers ($P = 0.011$ for $\text{mus81}\Delta \text{rad1}\Delta$ versus $\text{mus81}\Delta \text{rad1}\Delta \text{yen1}\Delta$), even though the crossover bands were barely visible in the cell population (Fig. 2c). Nine of 378 colonies analyzed from the $\text{mus81}\Delta \text{yen1}\Delta$ double mutant showed crossovers, a number significantly higher than for the triple mutant ($P = 0.0005$) and lower than for the wild type ($P = 0.032$). Thus, the same trends were found for the mutants when crossover products detected in populations and crossovers among surviving colonies were compared (Figs. 2b and 3b).

Physical analysis of recombination products in the cell populations revealed a decrease in formation of the 7-kb noncrossover band in the $\text{rad1}\Delta$ derivatives at 8 h (Fig. 2c,d). This decrease does not appear to be due to a delay in HO cleavage or strand invasion because crossover products were present at 8 h and strand-invasion intermediates detected by PCR were present at similar levels in the $\text{rad1}\Delta$ mutant and wild type (see below). The defect in formation of noncrossover products mirrors the reduced plating efficiency of the $\text{rad1}\Delta$ derivatives in response to the HO-induced DSB, suggesting that most of the lethality is due to loss of noncrossovers (Fig. 2b,d). To confirm a defect in formation of noncrossover products in the absence of Rad1, we measured the plating efficiency of strains containing a 1.2-kb $\text{ura3-HO-inc}$ donor on galactose-containing medium (Supplementary Fig. 2b). The $\text{rad1}\Delta$ strain with the 1.2-kb donor showed reduced plating efficiency on galactose as compared to the wild type ($P = 0.0005$), and the plating efficiency of the triple mutant was reduced to 0.11 ($P = 0.0001$ as compared to $\text{rad1}\Delta$). Because >99% of the products recovered from strains with the 1.2-kb substrate are noncrossover$^5$, the reduced viability of the $\text{rad1}\Delta$ mutants with the short substrate is consistent with loss of noncrossover recombinants. Using primers (P2 and P3) to monitor strand-invasion intermediates (as well as rare crossover products and unprocessed flap intermediates), we found that the $\text{rad1}\Delta$ derivatives showed no defect, indicating that the requirement for Rad1-Rad10 is subsequent to strand invasion and extension of the invading 3′ end by DNA synthesis (Supplementary Fig. 2c).

**Rad1-dependent formation of ectopic joint molecules**

If Rad1-Rad10 functions redundantly with Mus81-Mms4 and Yen1 to resolve recombination intermediates, we would predict a further increase in the accumulation of joint molecules in the triple mutant as compared with the $\text{mus81}\Delta \text{yen1}\Delta$ double mutant. Alternatively, if Rad1-Rad10 creates a joint molecule by cleaving the captured D-loop intermediate at the homology-heterology barrier, which is subsequently resolved by Mus81-Mms4 or Yen1, then the $\text{rad1}\Delta$ mutation should suppress the accumulation of ectopic joint molecules in the $\text{mus81}\Delta \text{yen1}\Delta$ double mutant. To distinguish between these possibilities, we used neutral two-dimensional gel electrophoresis to identify joint molecules during homology-dependent repair of the 5.6-kb repeat substrate (Fig. 4). The analysis was performed with nocodazole-treated (G2-M arrested) cells to avoid replication intermediates, but at later times cells broke through the arrest and resumed cycling, resulting in visible Y-shaped replication intermediates (Fig. 4c,d).

Two distinct branched DNA species were observed that were identified as the chr. II-V ectopic joint molecule (19.3 kb) and chr. V-V intersister joint molecule (14 kb) (IS-JM) on the basis of

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**Figure 3** Absence of crossovers from survivors of the $\text{mus81}\Delta \text{rad1}\Delta \text{yen1}\Delta$ mutant. (a) Genomic DNA from pools of five independent survivors was digested with ApaLI and PvuII and analyzed by Southern blot hybridization. Individual clones from pools that exhibited CO products were analyzed to verify formation of reciprocal COs. For simplicity, the null alleles are written as $\text{mus81}\Delta$, $\text{rad1}\Delta$ and $\text{yen1}\Delta$ in the figure. (b) Percentage COs from independent survivors of the indicated strains. * denotes $P$ value <0.05; ** denotes $P$ value <0.001 by Fisher’s exact test. (c) Left, schematic showing the sizes of parental (and NCO) and translocation products formed by reciprocal exchange between the $\text{ura3}$ repeats (white boxes). Probes specific for chr. II or V were designed to hybridize to sequences on the opposite chromosome arm. Right, pulsed-field gel electrophoresis to separate intact chromosomes from one NCO and three independently derived CO products from wild type. The left gel shows hybridization of the blot with the chr. V probe, and the right gel shows the chr. II probe.

**Figure 4** Joint molecules accumulate in the $\text{mus81}\Delta \text{yen1}\Delta$ double mutant. (a) Schematic showing the sizes of ApaLI-PvuII restriction fragments for the 5.6-kb $\text{ura3}$ repeats and expected intersister joint molecule (IS-JM), chr. II-V joint molecule and branched molecules resulting from HO cleavage of the IS-JM or intersister single-end invasion (IS-SEI) intermediates. Joint molecules could contain one or two Holliday junctions (one X shown for simplicity). (b) Cartoon of two-dimensional gel showing the migration of joint molecules and Y-shaped arcs; the open triangle marks the position of the 5.6-kb joint molecule, the filled triangle the position of the IS-JM and the open arrowheads the apparent IS-SEIs. (c) Time course following HO induction for wild-type, $\text{mus81}\Delta \text{yen1}\Delta$ and $\text{mus81}\Delta \text{rad1}\Delta \text{yen1}\Delta$ strains. For simplicity, the null alleles are written as $\text{mus81}\Delta$, $\text{rad1}\Delta$ and $\text{yen1}\Delta$ in the figure. (d) Fluorescence-activated cell-sorting profiles of the strains used for two-dimensional gel analysis; Asyn refers to the asynchronous population and Noco to nocodazole-arrested cells.
size and use of hybridization probes specific to each locus (Fig. 4c and Supplementary Fig. 3). Detection of the IS-JM indicates that HO cleavage was asynchronous, and one broken chromatid engaged with the uncut sister chromatid even though HO was continuously expressed during the time course. We presume that both sisters are eventually cut, forcing ectopic repair. Very faint spots corresponding to joint molecules were detected in the wild-type strain, suggesting that joint molecules are rapidly resolved. The IS-JM accumulated to much higher level (2.2% of total DNA) in theade2-∆mutants, consistent with an important role for S. pombe mus81

Rad1 is not required for LOH between chromosome homologs

If Rad1 is only required for recombination events that involve processing of branched structures formed at heterology boundaries, then the rad1Δ diploid should be proficient for crossovers between chromosome homologs. To test this, we used a previously described genetic assay to distinguish between noncrossover and crossover products induced by a site-specific DSB at theade2locus in diploid cells (Fig. 5a)23. In this system, an I-SceI–induced DSB at theade2-1allele is repaired from the homolog bearing a frame-shift mutation located 950 bp away from theade2-1allele (ade2-n). The diploid has dominant drug-resistance heterozygous markers 150 kb centromere distal toade2(HphandNat) and markers on the other chromosome arm (MET22andURA3) to distinguish LOH by mitotic recombination from chromosome loss. In these strains, I-Scelis under the transcriptional control of theGAL1promoter. Induction of I-Sclexpression results in high-frequency recombination to generate Adeneut (short tract gene conversion) or Ade−(long-tract gene conversion) recombinants. Ade−events due to long-tract gene conversion are distinguished from nonrecombinants (cells in which I-Scel was not induced or repair occurred between sister chromatids) by a reinduction assay23. Red-white–sectored colonies result from G2 repair of one broken chromatid by short-tract gene conversion and repair of the other broken chromatid by a long-tract gene conversion event (Fig. 5b). This class of colonies, representing 36% of the wild-type recombinants, is indicative of cells that were in G2 at the time the cells were plated and is the most informative to the mechanism of recombination, as both products of the recombination event are recovered in the two halves of the colony. A crossover associated with repair of one of the broken chromatids is detected by reciprocal LOH of theHphandNatmarkers, whereas BIR results in nonreciprocal LOH (Fig. 5c).

I-Scel was induced in liquid culture for 1–3 h, glucose was added to the cells and appropriate dilutions were plated onto medium with glucose to repress expression of the nuclease. There was no increase in DSB-induced chromosome loss in therad1Δmutant, indicating proficient homology-dependent repair, and the distribution of recombinants among the red-white–sectored colonies was the same
as for the wild type (Fig. 5d). In contrast to the ectopic system, the rad1 Δ mutation did not decrease crossovers by itself (P = 0.96) or in the mus81 Δ background (P = 0.66). Notably, the percentage of crossover events between homologs was reduced in the mus81 Δ mutant as compared with the wild type (P = 0.003) and the rad1 Δ mutant (P = 0.004), whereas crossovers between repeats were unaffected by the mus81 Δ mutation (Fig. 2). Crossovers between homologs were greatly reduced in the mus81 Δ yen1 Δ mutant and were lower than in the mus81 Δ rad1 Δ mutant (P = 0.0001). The mus81 Δ rad1 Δ yen1 Δ triple mutant showed poor vegetative growth, and only a low yield of red-white–sectored colonies was recovered from several independent trials (Supplementary Fig. 4). Although no crossovers were recovered from the triple mutant, this was not significantly different from the low number recovered from the mus81 Δ yen1 Δ double mutant (P = 0.09) (Supplementary Table 1). We cannot exclude the possibility that Rad1 has a minor role in the formation of crossovers between chromosome homologs but propose that it is more important for recombination between dispersed repeats.

**DISCUSSION**

We propose the following model for Rad1-Rad10 function in DSB-induced recombination between ectopic sequences (Fig. 6). After Rad51-dependent strand invasion to generate a D loop, the 3’ end is extended by DNA synthesis. If the DNA synthesis tract were short, then, following displacement of the invading strand, the nascent ssDNA would be able to pair with the other end of the break to complete repair by fill-in DNA synthesis and ligation. If the nascent strand were extended beyond the shared homology, then an unpaired flap would be generated after annealing of the displaced strand to the other side of the break. We suggest that Rad1-Rad10 would cleave the unpaired flap to allow completion of repair to form noncrossover products. This mechanism is similar to the single-strand annealing mechanism to repair DSBs between direct repeats34. A role for Rad1-Rad10 in unpaired flap removal was previously suggested to explain the defect in gene conversion between nontandem direct repeats in Ercc1−/− hamster cells44. Unpaired flaps would be more likely to form between sequences with short homology, and this could explain the reduced repair efficiency of the rad1 Δ derivatives with the 1.2-kb substrate as compared with the 5.6-kb substrate (Supplementary Fig. 2).

Capture of the D loop by the ssDNA formed at the other break end, followed by gap filling and ligation, would result in formation of a dHJ and subsequent dissolution by STR to generate noncrossover products (Fig. 6). The dHJ could also be cleaved byendonucleases to form crossover or noncrossover products, but recent studies indicate that this mechanism is inefficient in mitotic cells17. Mus81-Mms4 could directly cleave the captured D loop before ligation, or Rad1-Rad10 could cut if resection had proceeded beyond the heterology boundary to create a single-stranded region adjacent to the branch point. Cleavage of the D loop, coupled with Mus81-Mms4 cutting of the nicked HJ, would result in a CO product. Alternatively, clipping of the D loop followed by gap filling and ligation would generate an sHJ intermediate that could not be dissolved by STR and would require Mus81-Mms4 or Yen1 for resolution. Intermediates are shown in blue boxes, NCO products in green boxes and CO products in brown boxes.
To explain the high-frequency plasmid integration observed in the mus81A yen1A double mutant, we suggest that a SHJ intermediate covalently linking the plasmid to chr. XII persists and is not recognized as aberrant during mitosis because the plasmid lacks a centromere and is resolved by replication through the Holliday junction in the next cell cycle, resulting in episomal and integrated plasmid products (Supplementary Fig. 6). Replication of a DHJ intermediate would not result in plasmid integration. This mechanism might explain the higher percentage of crossovers observed during plasmid gap repair as compared with ectopic recombination between chromosomal repeats. Resolution by replication could also explain why only a minor defect was observed for the mus81A yen1A mutant when cells were transformed with a plasmid containing a SHJ. The dependence on Rad1, and high frequency of plasmid integration, might also be explained if there were less of a constraint on second-end capture during plasmid gap repair than during chromosomal DSBR.

Unexpectedly, ectopic crossovers were recovered from the mus81A yen1A double mutant even though unresolved joint molecules persist in this strain (Figs. 3 and 4). The covalently linked nonsister chromatids would be expected to co-segregate to the same daughter cell in 50% of mitoses, and the SHJ connecting them might be replicated in the next cell cycle, resulting in products indistinguishable from crossovers (as suggested for plasmid integration in the absence of Mus81 and Yen1). Replication has also been proposed to explain the resolution of Holliday junction–containing chromosomal DNA in E. coli ruvB mutants. Breakage of the joint molecule during mitosis and repair of fragments by break-induced replication in the next cell cycle would lead to inviable products and might contribute to the reduced viability of the mus81A yen1A mutant following DSBR induction. Alternatively, it is conceivable that, after cleavage of the D loop by Rad1-Rad10, the branch point could migrate back to the other heterology boundary and Rad1-Rad10 could cut again, yielding a crossover (Supplementary Fig. 6).

In summary, we have identified a new function for the Rad1-Rad10 nuclease in promoting DSBR-induced crossovers between dispersed repeats by converting a strand-invasion intermediate to an intermediate that can only be resolved by Mus81 and Mms4 or Yen1. The function of XPF-ERCC1 in heterologous flaps is conserved in mammals, suggesting that the role in crossover formation is also likely to be conserved. Mammals have a much larger repertoire of repeated sequences than do budding yeast, raising the possibility that XPF-ERCC1 has a significant role in the formation of gross chromosomal rearrangements leading to genomic disorders in higher eukaryotes. Given the potential of structure-selective nucleases to generate detrimental crossovers in mitotic cells, it is important for their activities to be highly regulated during the cell cycle and to be used as a last resort when less mutagenic options are available.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Supplementary information is available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

G.M., C.K.H., M.K. and L.S.S. designed experiments, and L.S.S. wrote the paper. Experiments in Figure 1 and Supplementary Figure 1 were carried out by A.E.L. and C.K.H., in Figures 2, 3 and 5, and Supplementary Figure 2 by G.M. and A.E.L., and in Figure 4 and Supplementary Figure 3 by G.M.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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ONLINE METHODS

Yeast strains. *S. cerevisiae* strains used in this study are listed in Supplementary Table 2. All strains are in W303 background and were generated by crossing strains with different recombination reporters to strains with mus81::KanMX6, rad1::LEU2 or yen1::HIS3 alleles 23,35,56.

Plasmid DNA gap-repair assay. The gap-repair plasmid pSB110 and transformation protocol were described previously 35. The frequency of gap repair is the number of Ura+ transformants per microgram of linearized DNA transformed divided by the number of Ura+ transformants per microgram of circular plasmid DNA transformed. In tests for mitotic stability, all of the Ura+ transformants were picked from one region of a plate, transferred to water-filled 96-well microtiter plates and spotted onto synthetic complete medium lacking uracil (SC–Ura). The cells were grown for 3 d, then replica plated to rich medium (1% yeast extract, 2% peptone, 2% glucose; YPD) and grown for 2 d to allow loss of the plasmid. The cells were then replica-plated to medium containing 5-fluoroorotic acid (5-FOA). Confluent growth on 5-FOA indicated that the Ura+ phenotype was mitotically unstable owing to plasmid repair without integration.

DSB-induced ectopic recombination assay. Plating efficiency, Southern blot analysis and PCR methods to analyze recombination intermediates were as described previously 27,56. Cells were pregrown with lactate as a carbon source before galactose induction 27,56. Cells were plated at the 8-h time point, following HO induction for analysis of survivors. Cultures (1 ml) of five independent survivors were pooled for DNA extraction and Southern blot analysis. If crossover products were detected in a pool, individual clones form the pool were analyzed to distinguish between crossover and noncrossover recombinants. Independent noncrossover and crossover recombinant clones were analyzed by pulsed-field gel electrophoresis (Bio-Rad CHEF II system) followed by Southern blot hybridization using probes specific for chr. II or chr. V. Between 20 and 40 independent colonies obtained after 8-h HO induction were also analyzed by PCR and BamHI digestion to calculate the number of recombinants for each of the strains used in Figure 3. PCR using primers P2 and P3 was used to detect strand-invasion intermediates, crossover products and noncrossover products with an unrepair heterologous flap. Template (50 ng) of the indicated strains isolated from cells at different times after HO induction was amplified for 27 cycles.

Neutral two-dimensional gel electrophoresis. Samples for neutral two-dimensional gel electrophoresis were obtained from 30-ml aliquots of cultures arrested with nocodazole (15 µg/ml) and induced with 2% galactose. Cells were embedded in agarose and lysed as described previously 57. Agarose plugs were melted and treated with β-agarase after digestion with ApaLI-PvuII, and the genomic DNA was precipitated. Three plugs were combined to load each lane of the first-dimension gel. Conditions for two-dimensional gels were as described 57. Filters were hybridized with URA3-specific, chr. II–specific or chr. V–specific probes.

DSB-induced ade2 recombination assay. I-SceI induction and analysis of recombinant colonies were as described previously 23. If both broken chromatids are repaired by short-tract gene conversion, a solid white colony is produced, two long-tract gene conversions produce a solid red colony, and red-white–sectored colonies are due to one short- and one long-tract conversion. Because the sectored colonies are diagnostic of two independent repair events, a colony that retained heterozygosity for Nat and Hph was scored as two noncrossover events; similarly, a sectored colony with reciprocal LOH was scored as one noncrossover and one crossover event. In a previous study, each sectored colony was scored as only one event, and this resulted in a larger number of crossovers than scored with the current method 23. Crossover events are only detected if the two recombinant chromatids segregate to opposite poles at mitosis 3, thus the numbers presented derive from doubling the number of crossover events and subtracting an equivalent number from the noncrossover class (Fig. 5d). Statistical significance for the distribution of recombination events between given strains was calculated by Fisher’s exact test (Supplementary Table 1). Independent inductions were performed at least three times for each strain.

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