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Mutations affecting a putative MutL α endonuclease motif impact multiple mismatch repair functions

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Abstract

Mutations in DNA mismatch repair (MMR) lead to increased mutation rates and higher recombination between similar, but not identical sequences, as well as resistance to certain DNA methylating agents. Recently, a component of human MMR machinery, MutL α , has been shown to display a latent endonuclease activity. The endonuclease active site appears to include a conserved motif, DQHA(X)₂E(X)₄E, within the COOH-terminus of human PMS2. Substitution of the glutamic acid residue (E705) abolished the endonuclease activity and mismatch-dependent excision in vitro. Previously, we showed that the *PMS2-E705K* mutation and the corresponding mutation in *Saccharomyces cerevisiae* were both recessive loss of function alleles for mutation avoidance in vivo. Here, we show that mutations impacting this endonuclease motif also significantly affect MMR-dependent suppression of homeologous recombination in yeast and responses to S_n1-type methylating agents in both yeast and mammalian cells. Thus, our in vivo results suggest that the endonuclease activity of MutL α is important not only in MMR-dependent mutation avoidance but also for recombination and damage response functions.

Keywords

Mismatch repair; MutL α ; Endonuclease; Recombination; Methylation tolerance

1. Introduction

DNA mismatch repair (MMR) is a conserved process, which repairs mismatches generated during replication, by chemical damage and as “heteroduplex” intermediates during recombination [1–4]. The MMR system also suppresses recombination between similar but non-identical sequences, homeologous recombination [2,5]. Furthermore, in eukaryotes MMR plays a role in responses to certain types of DNA damage [1,6–8], in particular by S_n1-type DNA methylating agents [1,7–10]. Not surprisingly, MMR defects lead to a mutator phenotype, which in humans and mice is associated with increased cancer susceptibility, most notably hereditary nonpolyposis colon cancer (HNPCC) [1,11–15].

The minimal human MMR system has been reconstituted in vitro with purified recombinant proteins, MutS α , MutL α , PCNA, RFC (the “clamp loader”), EXO1, RPA (HMGB1), polymerase δ and DNA ligase I [16–18]. Similar to cell-free extracts, the in vitro repair is directed by a strand discontinuity (a nick or gap) that can be located either 5′ or 3′ of the mismatch. After recognition of mismatch, the mismatch-containing strand is degraded exonucleolytically from the pre-existing nick or gap and subsequently the resulting single-

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stranded gap is resynthesized and ligated. One of the essential components of the MMR machinery is MutL α , a heterodimer of MLH1 and PMS2 (Pms1 in yeast) [1–3,17,19]. Data from in vitro studies indicate that MutL α is recruited to the heteroduplex DNA in a MutS α - and ATP-dependent fashion and acts as an ATPase that coordinates protein conformational changes which are important for MMR [20–23]. The in vitro studies further uncovered some of the specific MutL α functions, including enhancement of mismatch-dependent excision and participation in termination of excision upon mismatch removal [16–18].

Recently, biochemical studies of reconstituted human and yeast MMR have demonstrated a latent endonuclease activity within MutL α [24, and personal communication, P. Modrich and T. Kunkel]. This endonuclease activity is activated by MutS α bound to the mismatch, ATP, PCNA and RFC. As a result, MutL α cleaves the discontinuous strand largely on the distal side of the mismatch, thus generating a mismatch-containing DNA fragment spanned by two nicks. Subsequently, this fragment is degraded by MutS α -activated EXO1. This endonuclease activity of MutL α can account for the observation that EXO1, which only possesses 5'–3' activity, is the only exonuclease required for the reconstituted in vitro MMR system, regardless of the position of the initiating nick with respect to the mispair [24,25].

The endonuclease active site of human MutL α includes a conserved motif, DQHA(X)₂E (X)₄E, within the COOH-terminus of PMS2 [24]. Substitution of either the aspartic (D699) or the glutamic acid residue (E705) abolished the endonuclease activity and mismatch-dependent excision [24]. We recently reported that the *PMS2-E705K* mutation and the corresponding mutation *pms1-E707K* (previously referred to as *pms1-E738K*) in the budding yeast *Saccharomyces cerevisiae* were both recessive mutations that crippled MMR-dependent mutational avoidance in vivo [26]. Here, we address the importance of the conserved endonuclease motif for additional MMR-dependent functions in budding yeast and mammalian cells. We show that mutations within the endonuclease motif significantly affect MMR-dependent suppression of homeologous recombination in yeast and responses to S_n1-type methylating agents in both yeast and mammalian cells. In addition, these findings have implications for the mechanisms of the damage response and recombination suppression by MMR.

2. Materials and methods

2.1. Media and growth conditions

All media were prepared as described [27] except that synthetic media contained increased leucine (60 mg/l). Growth and sporulation were at 30 °C. Sporulation of diploid cells and tetrad dissections were performed as described [28,29].

SJR1392 and derivatives were grown in YEPGE (2% glycerol, 2% ethanol and supplemented with 500 μ g/ml adenine hemi-sulfate and 30 μ g/ml tryptophan) [28]. For selection of His⁺ recombinants, S.D. medium lacking histidine was supplemented with 2% glycerol, 2% ethanol and 2% galactose as carbon sources (SGGE-His) [28].

2.2. Strain constructions

All yeast strains are derivatives of a *RAD5 CAN1* W303-1B unless otherwise noted (see Table 1) [30,31]. W303 derivatives (*CAN1*, *his1-7*, *his7-2*, *hom3-10*, *pms1 Δ* , *pms1-E707K* and *rad52 Δ*) were described previously [29,32].

MGT1 disruption was constructed in haploid strains using a PCR-based gene disruption method [33] utilizing *TRP1* as a selective marker and the following primers, with upper-case letter sequences complementary to *MGT1* sequences and lowercase denoting selective marker sequences: *MGT1*-H1 (5'-AAAAAAATTG AAAACGGTCG CATT TTTGAT

CTAAATGGAC CAACGgagcagattgtactgag-3') and *MTG1*-H2 (5'-ACATAACTATTTCTTATGTTTATTTTCCTAAAATCCTTTATCCAA tgtgcggtattcacacc-3'). The disruption was confirmed by PCR.

The introduction of *pms1Δ* and *pms1-E707K* into SJR1392 were performed as described previously [26,29].

2.3. Rate measurements and statistical analyses

The method of median was used to calculate the mutation rate [34]. Data from at least 20 independent cultures were used for each rate determination. Briefly, individual colonies from SJR1392 and its derivatives were grown in YEPGE medium for 3 days [28]. Aliquots of appropriate dilutions were plated onto synthetic complete medium (to determine viability of the cells), medium lacking histidine and glucose, but containing galactose, ethanol and glycerol (to select for homeologous recombinants) and medium lacking lysine (to select for homologous recombinants). Statistical analyses were performed using Prism 3.0 Software (GraphPad Software Inc.).

2.4. MNNG sensitivity by cytotoxicity and “spotting” assays

Sensitivity to a pulse of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG; Sigma Chemical Co., St. Louis, MO) was determined by exposing exponentially growing yeast cells to varying amounts of MNNG for 45 min followed by plating of appropriate dilutions on YPD. Surviving colonies were counted after 3 days of incubation at 30 °C. For all strains, a plot of ln(survival %) versus dose yielded a straight line and an LD₅₀ was calculated as $-\ln(2)/\alpha$, where α is the slope of the straight line.

For spotting assays, relevant yeast strains were grown overnight to saturation in YPD, serially diluted (1:5) and spotted with a 48-prong replicator onto synthetic complete medium (CSM) and CSM containing 0.35 μM MNNG plates and grown at 30 °C for 2 days.

For microscopy, logarithmically growing liquid yeast cultures were exposed to 0.5 μM MNNG for 45 min followed by washing and resuspending of the yeast culture in fresh media. Aliquots of each culture were taken at appropriate time points. Cells were harvested by centrifugation and fixed with methanol/acetic acid (3:1 ratio) for 0.5 h on ice and stained with 4',6-diamino-2-phenylindole (DAPI, Accurate Biochemicals and Scientific Corp., Westbury, NY) for 5 min at room temperature. Images were collected using a Zeiss Axiovert LSM5 Pascal laser-scanning microscope. At least 100 cells were counted for each sample and each experiment was done in duplicate.

2.5. Culturing of mouse cell lines and 6-tg treatment

Spontaneously-immortalized mouse embryonic fibroblast (MEF) cell lines used in this study were derived from *Pms2*^{-/-} C18 cells and grown as previously described [22,26,35].

Sensitivity to a “pulse” treatment of 6-tg (2-amino-6-mercaptopurine; Sigma Chemical Co.) was determined by exposing exponentially growing cells to different concentrations of 6-tg diluted into complete medium. Following a 24 h treatment at 37 °C, cells were rinsed once with 5 ml of PBS and refed with complete medium. After 8 days, cell colonies were fixed in 30% ethanol 50% methanol, stained with 0.25% methylene blue and counted. For each dose, three 100-mm plates were analyzed, seeded with either 300, 600 or 1000 cells. Each cell line was tested in three independent experiments, and the colony-forming units (CFUs) at each dose were averaged. For all strains, a plot of ln(survival%) versus dose yielded a straight line and an LD₅₀ was calculated as $-\ln(2)/\alpha$, where α is the slope of the straight line.

2.6. Western blot analysis

Whole cell lysates from cultured cells were prepared and 50 µg of total cellular protein were used for Western blot analysis as described previously [26,35]. hPms2 and mMsh6 proteins were detected with monoclonal antibodies A16-4 and clone 44, respectively (BD Pharmingen).

3. Results and Discussion

Recent in vitro studies suggest that the endonuclease active site of human MutLα includes a conserved motif, DQHA(X)₂E(X)₄E [24], within the MLH1-interaction domain of PMS2 (Fig. 1a, [36]). Substitution of the first glutamic acid residue (E705) abolishes the endonuclease activity of human MutLα [24]. However, this substitution does not detectably affect either yeast Pms1/Mlh1 or human PMS2/MLH1 interactions, the stability of Pms1 or PMS2 [26] or interaction of MutLα with MutSα on a mismatch (personal communication, P. Modrich and T. Kunkel). Nevertheless, we cannot exclude the possibility that this substitution might affect other functions of MutLα such as interactions with unknown proteins. Our previous in vivo studies showed that the *E705K* mutation in human *PMS2* and the corresponding mutation *E707K* in the *PMS1* gene of *S. cerevisiae* were both recessive loss of function mutations for MMR spellchecker activity [26]. Please note that in contrast to our previous report [26], we now designate the relevant glutamic acid as residue 707 in accordance with the Stanford *S. cerevisiae* data base. Here, we have addressed the importance of this conserved endonuclease motif for additional MMR-dependent functions in budding yeast and mammalian cells.

3.1. pms1-E707K cripples MMR of different types of mispairs

Our previous study using a single mutation reporter (i.e., *hom3-10*) showed that the *pms1-E707K* allele elevated −1 bp frameshifts to a level similar to a *pms1Δ* strain [26]. To gain additional insight into the role of the endonuclease motif for MMR-dependent mutation avoidance in yeast, we measured mutation in the *pms1-E707K* strain using four different mutator assays [29]: forward mutation to canavanine-resistance at *CAN1* that reports multiple types of mutations, *his1-798* (*his1-7*) reversion that reports base substitution (intragenic suppressor) mutations, *his7-2* reversion that reports +1 bp frameshifts in a run of 7A/T bp and *hom3-10* reversion that reports −1 bp frameshifts in a 7A/T run. Previous work demonstrated that elevation of mutation in each assay in a *pms1Δ* strain can be visualized by increased number of papillae on the appropriate selective media (Fig. 1b). As shown in Fig. 1b, the number of papillae observed with the *pms1-E707K* strain was similar to *pms1Δ* in each assay. Therefore, these results suggest that the endonuclease activity of MutLα is required for the repair of a variety of mispairs.

3.2. pms1-E707K impacts the suppression of homeologous recombination

The role of MMR proteins in the suppression of homeologous recombination is well-established in yeast, although the mechanism of suppression, including the role of MutLα, is not clear [2,5]. To address whether the endonuclease activity of MutLα is important for suppression of homeologous recombination, the *pms1-E707K* allele was introduced into the *PMS1* locus of a haploid yeast strain (SJR1392), which contains reporters that measure rates of homeologous versus homologous recombination between inverted repeats [28]. We measured the rates of homeologous recombination and homologous recombination by selecting for inversion events that reconstitute full-length *HIS3* and *LYS2* genes, respectively. As shown in Table 2, *pms1Δ* significantly increased the homeologous recombination rate, without elevating homologous recombination. Normalized to homologous recombination, the increase in the homeologous recombination was 24-fold in the *pms1Δ* mutant relative to *PMS1*.

Similarly, *pms1-E707K* elevated the homeologous recombination rate by 8-fold relative to the wild type. These results suggest that the endonuclease activity of yeast MutL α contributes to the suppression of homeologous recombination.

Genetic requirements of suppression of yeast homeologous recombination have been examined in great detail [2,5]. In particular, the suppressive role of MutL α seems less than that of MutS α , because *msh2 Δ* has a greater “elevating” effect on homeologous recombination than either *pms1 Δ* or *mlh1 Δ* [37–39]. Therefore, mismatch binding/recognition may be sufficient to trigger some level of recombination suppression in yeast. Although the exact role of MutL α in homeologous recombination suppression is unknown, our results with the *pms1-E707K* allele suggest that the endonuclease activity of MutL α is important. It is interesting to note that the effect of *pms1 Δ* allele on homeologous recombination appears to be greater than the *pms1-E707K* allele. Therefore, MutL α appears to contribute to the suppression of homeologous recombination by both endonuclease-dependent and -independent actions. We suggest that the endonuclease-dependent contribution may reflect events containing mismatched hDNA intermediates in which a single strand interruption 3' to the mismatch(es) provides an entry point for MMR-mediated suppression.

3.3. *pms1-E707K* (PMS2-E705) compromises the MMR-dependent DNA damage responses in yeast and mammalian cells

In mammalian cells, MMR also participates in cytotoxic and cell cycle arrest responses to certain classes of DNA damaging agents, most notably S_N1-type methylating agents [1,7–10]. Mammalian cells deficient in MMR show increased resistance to the cytotoxic effects of S_N1-type methylating agents, such as nitrosoguanidine (MNNG) and the methylation mimetic drug, 6-thioguanine (6-tg) [8,40–42]. Recently, a similar MMR-dependent response was demonstrated in yeast cells that were defective in both homologous recombination (*rad52 Δ*) and the methylguanine methyltransferase (*mgt1 Δ*) [43]. This is illustrated in Fig. 2a and b, which shows that whereas a *rad52 Δ mgt1 Δ* strain was highly sensitive to MNNG, the MMR defective *rad52 Δ mgt1 Δ pms1 Δ* strain was resistant.

To ask whether the endonuclease activity of MutL α is required for the damage response pathway in yeast, we compared the cytotoxic responses of the *rad52 Δ mgt1 Δ* , *rad52 Δ mgt1 Δ pms1 Δ* and *rad52 Δ mgt1 Δ pms1-E707K* strains to MNNG. As shown in Fig. 2a, even at relatively low doses, the *rad52 Δ mgt1 Δ pms1-E707K* strain displayed a level of resistance to MNNG similar to that observed for *rad52 Δ mgt1 Δ pms1 Δ* . This finding was further confirmed by calculating the LD₅₀ values (i.e., 50% survival) for each strain at a range of MNNG doses. Whereas the LD₅₀ of *rad52 Δ mgt1 Δ* strains was 0.25 μ M, the LD₅₀ of the *rad52 Δ mgt1 Δ pms1 Δ* and *rad52 Δ mgt1 Δ pms1-E707K* strains were 1.8 and 1.5 μ M, respectively. These observations suggest that the endonuclease activity of yeast MutL α is required for the cytotoxic response to S_N1-type methylating agents.

In cultured mammalian cells, MMR-dependent responses to S_N1-type methylating agents are associated with G₂ cell cycle arrest [8,35,44]. To determine whether yeast show a similar cell cycle arrest, we microscopically monitored logarithmically growing *rad52 Δ mgt1 Δ* , *rad52 Δ mgt1 Δ pms1 Δ* and *rad52 Δ mgt1 Δ pms1-E707K* strains following treatment with 0.5 μ M MNNG, a concentration that results in ~70% killing of the *rad52 Δ mgt1 Δ* cells with little effect on either the *rad52 Δ mgt1 Δ pms1 Δ* or *rad52 Δ mgt1 Δ pms1-E707K* strain (see Fig. 2b). Cells were harvested at various times, fixed and stained with DAPI to visualize nuclear morphology. Within 2 h of MNNG treatment, the *rad52 Δ mgt1 Δ* cells accumulated with a nuclear and budding morphology characteristic of G₂/M arrested yeast (see Fig. 3). In contrast, *rad52 Δ mgt1 Δ pms1 Δ* and *rad52 Δ mgt1 Δ pms1-E707K* cells both grew normally in MNNG. These results indicate that the response to MNNG in yeast is associated with a G₂/M arrest, consistent with observations in mammalian cells.

Previous studies showed that *Pms2*-null kidney [45] and mouse embryonic fibroblasts cells (Buermeier and Liskay, unpublished results) were resistant to the cytotoxic effects of 6-thioguanine (6-tg), a S_n1 -type methylation mimetic drug [46,47]. Therefore, we determined the colony-forming ability of *Pms2*^{-/-} null mouse embryonic fibroblasts (MEF) stably expressing empty vector, wild type hPMS2 or hPMS2-E705K after 24 h exposure to different concentrations of 6-tg (Fig. 4a). Two cell lines that expressed hPMS2 at different levels were highly sensitive to 6-tg relative to the *Pms2*-null cell lines (Fig. 4, compare LD₅₀ of 0.28 μ M 6-tg for hPMS2 lines versus LD₅₀ of 1.33 μ M 6-tg for the *Pms2*-null), indicating that expression of wild type *Pms2* within this range is sufficient for the normal response to 6-tg. Similar to the *Pms2*-null cells, cells expressing hPMS2-E705K were highly resistant to 6-tg, showing an LD₅₀ of 1.31. These results suggest that, similar to budding yeast, the endonuclease activity of human MutL α is required for the MMR-dependent DNA damage response to S_n1 -type methylating agents.

Two contrasting, but not mutually exclusive models, have been proposed for the MMR-dependent response to S_n1 -type methylating agents in mammalian cells [3,10,42,48]. The first model, termed “futile repair”, invokes repeated cycles of MMR on O⁶meG/T formed during replication. Repeated cycles fail to remove damage in the template strand that in turn may lead to double strand breaks [8]. In contrast, in the “direct signaling” model MMR protein complexes bound to O⁶meG/T mispairs act as direct sensors to signal for cell cycle checkpoint arrest and/or apoptosis [3,10,42,48].

Support for the futile cycling model comes from several studies including in vitro results showing reiterative MMR excision cycles on O⁶meG/T containing plasmid substrates [49]. In contrast, support for direct signaling comes in part from a “separation-of-function” allele of mouse *Msh6* that impacts spellchecking with little or no effect on the MMR-dependent DNA damage response [50]. The signaling model is also supported by the demonstration that MutS α /MutL α complexes are required for the recruitment of ATR–ATRIP to methylation damage sites (O⁶meG/T mispairs) and subsequent ATR kinase activation [51]. The results reported here appear consistent with futile repair because a defective endonuclease can be expected to impede repeated cycles of MMR-dependent excision.

In summary, by altering a conserved motif in both yeast *Pms1* and human PMS2, we asked whether the endonuclease of yeast and human MutL α is important for several MMR-dependent functions. We draw several conclusions from our study. First, the MutL α endonuclease of yeast is required for the repair of a variety of different types of mispairs. Second, the MutL α endonuclease of yeast contributes to the suppression of homeologous recombination. And third, in both yeast and mammalian cells, the endonuclease activity of MutL α is required for the MMR-dependent response to DNA methylation damage.

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Fig. 1.

(a) Pms1/2 domains and location of E707/E705. The ATPase domain, the Mlh1-interacting domain and a motif critical for MutLα endonuclease activity are indicated by hatched, black and grey boxes, respectively. The “mutated” E707/E705 residue is indicated by an arrow. Numbers correspond to the amino acid position in the protein in accordance with the current data bases. (b) Papillation phenotypes of the wild type, *pms1Δ*, *pms1-E707K* strains. The relative mutator effects in wild type, *pms1Δ*, *pms1-E707K* strains were detected by monitoring forward mutation at *CAN1* (multiple types of mutations) vs. reversion at *his1-7* (intragenic missense suppressor mutations), *his7-2* (+1 bp frameshifts in a stretch of 7A/T bp) or *hom3-10* (−1 bp frameshifts in a stretch of 7A/T bp) by replica plating onto appropriate selective media. It should be noted that *pms1Δ* strains contain *ade2-1* that results in solid red colored papilliae, whereas *pms1-E707K* strains contain a *ade2-9G* (+1 bp frameshift in a stretch of 9G/C bp) reporter that gives rise to red/white papilliae due to DNA polymerase slippage in the 9 G/C run.

Fig. 2. MNNG-induced killing of *rad52 Δ mgt1 Δ* , *rad52 Δ mgt1 Δ pms1 Δ* and *rad52 Δ mgt1 Δ pms1-E707K* yeast strains: (a) comparison of MNNG responses for wild type, *mgt1 Δ* , *rad52 Δ* and *pms1* mutants in combinations as measured by a serial dilution spot test. Late-log phase cells were spotted on CSM and CSM + 0.35 μM MNNG plates at 5-fold serial dilutions as described in the Section 2. (b) MNNG survival curves. *mgt1 Δ* (■), *rad52 Δ mgt1 Δ pms1 Δ* (▲), *rad52 Δ mgt1 Δ pms1-E707K* (●) and *rad52 Δ mgt1 Δ* (◆) strains were exposed for 45 min to increasing amounts of MNNG and survival was determined. Error bars represent standard error of the mean. LD₅₀ values for *mgt1 Δ* , *rad52 Δ mgt1 Δ pms1 Δ* , *rad52 Δ mgt1 Δ pms1-E707K* and *rad52 Δ mgt1 Δ* strains were 9.7, 1.8, 1.5 and 0.25 μM MNNG, respectively.

Fig. 3.

Cell cycle arrest phenotype of *rad52Δ mgt1Δ* mutant strains after exposure to MNNG. After 45 min exposure to 0.5 μ M MNNG, cells from *rad52Δ mgt1Δ*, *rad52Δ mgt1Δ pms1Δ* and *rad52Δ mgt1Δ pms1-E707K* cultures were collected every hour, fixed and stained with DAPI: (a) after 2 h, *rad52Δ mgt1Δ* accumulated as large-budded cells with a single nucleus and (b) quantification of cell cycle arrest after MNNG exposure. Distribution of cells from *rad52Δ mgt1Δ*, *rad52Δ mgt1Δ pms1Δ* and *rad52Δ mgt1Δ pms1-E707K* cultures after exposure to MNNG are depicted as percentages. Cells were divided into categories as cells with no buds, small buds, large buds and divided cells. For each time point, at least 100 cells were counted in two separate experiments.

Fig. 4.

Survival of MEFs after treatment with 6-tg: (a) 8 days after a 24 h exposure to 6-tg, CFU were determined for *Pms2*-deficient (♦), hPMS2-E705K expressing (▲) and low and high hPMS2-expressing (■, ●) MEF cells. Error bars represent standard error of the mean. LD₅₀ values were 1.33, 1.31, and 0.28 μM 6-tg for *Pms2*-deficient, hPMS2-E705K expressing and low hPMS2-expressing MEF cells, respectively. (b) Expression of hPMS2 and hPMS2-E705K in stably-transfected MEFs. To determine the level of hPMS2-E705K vs. wild type hPMS2 expression levels in MEFs, whole cell lysates were prepared and compared by Western analysis. Endogenous Msh6 expression was used to normalize hPMS2 expression.

Table 1

Strains used in this study

Strain	Genotype	Source
W1558-4A	<i>MATaade2-1 CAN1 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5</i>	[31]
NEY915	W1558 <i>mgt1::TRP1</i>	This study
NEY978	W1588 <i>mgt1::TRP1 rad52::HIS5</i>	This study
NEY981	W1588 <i>mgt1::TRP1 rad52::HIS5 pms1::TRP1</i>	This study
NEY1012	W1588 <i>mgt1::TRP1 rad52::HIS5 pms1-E707K</i>	This study
NEY524	<i>MATaade2-1 CAN1 his1-7 hom3-10 leu2-3,112 trp1-1 ura3-1 RAD5</i>	[29]
NEY442	NEY524 <i>pms1::TRP1</i>	[29]
NEY1349	NEY524 <i>pms1-E707K ade2-9G</i>	This study
NEY570	<i>MATaade2-1 CAN1 his7-2 hom3-10 leu2-3,112 trp1-1 ura3-1 RAD5</i>	[29]
NEY662	NEY524 <i>pms1::TRP1</i>	[29]
NEY1350	NEY524 <i>pms1-E707K ade2-9G</i>	This study
SJR1392	<i>MATaade2-101 oc his3Δ 200 lys2Δ RV::hisG trp5 Δ::kan ura3(Nhe)-[HIS3::intron::cβ2/cβ7(91%)]-ura3(Nhe) leu2(K)-[lys2Δ3'-lys2Δ5']-LEU2</i>	Sue Jinks-Robertson
NEY644	SJR1392 <i>pms1::URA3</i>	This study
NEY1351	SJR1392 <i>pms1-E707K</i>	This study

Table 2
Effect of *pms1-E707K* on homeologous recombination

Strain	Homologous (Lys ⁺ , × 10 ⁻⁶)	Homeologous (His ⁺ , × 10 ⁻⁶)	His ⁺ /Lys ⁺	
<i>PMS1</i>	5.0 (4.1–5.9)	0.07 (0.051–0.089)	0.014	[1]
<i>pms1Δ</i>	7.4 (6.2–8.5)	2.42 (1.8–3.0)	0.33	[24]
<i>pms1-E707K</i>	12.6 (8.6–17)	1.35 (1.1–1.6)	0.11	[8]

() 95% Confidence intervals; [] fold effects relative to *PMS1*.