Different Roles of Eukaryotic MutS and MutL Complexes in Repair of Small Insertion and Deletion Loops in Yeast

Nina V. Romanova¹, Gray F. Crouse¹,2*

¹ Department of Biology, Emory University, Atlanta, Georgia, United States of America, ² Winship Cancer Institute, Emory University, Atlanta, Georgia, United States of America

Abstract

DNA mismatch repair greatly increases genome fidelity by recognizing and removing replication errors. In order to understand how this fidelity is maintained, it is important to uncover the relative specificities of the different components of mismatch repair. There are two major mismatch recognition complexes in eukaryotes that are homologues of bacterial MutS proteins, MutSα and MutSβ, with MutSα recognizing base-base mismatches and small loop mispairs and MutSβ recognizing larger loop mispairs. Upon recognition of a mispair, the MutS complexes then interact with homologues of the bacterial MutL protein. Loops formed on the primer strand during replication lead to insertion mutations, whereas loops on the template strand lead to deletions. We show here in yeast, using oligonucleotide transformation, that MutSα has a strong bias toward repair of insertion loops, while MutSβ has an even stronger bias toward repair of deletion loops. Our results suggest that this bias in repair is due to the different interactions of the MutS complexes with the MutL complexes. Two mutants of MutLx, pms1-G882E and pms1-H888R, repair deletion mispairs but not insertion mispairs. Moreover, we find that a different MutL complex, MutLγ, is extremely important, but not sufficient, for deletion repair in the presence of either MutLx mutation. MutSβ is present in many eukaryotic organisms, but not in prokaryotes. We suggest that the biased repair of deletion mispairs may reflect a critical eukaryotic function of MutSβ in mismatch repair.

Introduction

DNA mismatch repair (MMR) is a major repair system in organisms ranging from bacteria to humans. The discovery that MMR defects cause the most common form of inherited colon cancer underscored the importance of this repair pathway to human health [1–6]. In eukaryotes, MMR involves recognition of mismatches created during replication by protein complexes that are homologues of bacterial MutS, followed by downstream processing events involving homologues of bacterial MutL [7–9]. There are two main recognition complexes, MutSα, a heterodimer consisting of Msh2 and Msh6 that recognizes base-base mismatches and small loops, and MutSβ, a heterodimer consisting of Msh2 and Msh3 that recognizes mainly loops [7–10].

The exact role that MutSβ plays in MMR is not clear. Loss of MutSβ causes only a weak mutator effect unless the assay is specific for insertion or deletion (in/del) mutations [11,12]. In general, there seems to be much less MutSβ protein than MutSα protein in yeast and human cells [13–15]; however, a recent report suggests that the relative amounts of MutSα and MutSβ vary in mouse tissues, with some tissues containing more MutSβ than MutSα [16]. A number of organisms such as Drosophila melanogaster and Caenorhabditis elegans apparently have no MutSβ although they have MutSβ [17]. Most analysis of MutSβ MMR function has tended to center on its repair of loops compared to the repair of base-base mismatches by MutSα. However, two early studies of MutSβ and microsatellite instability in yeast found a surprising difference in loop repair and loss of MutSβ compared to loss of MutSα [18,19]. For example, using an assay for dinucleotide repeat slippage, Sia et al. found more insertions than deletions in wild-type cells, whereas complete loss of MMR resulted in approximately equal numbers of insertions and deletions; strikingly, cells containing only MutSα had many more deletions than insertions whereas cells containing only MutSβ had many more insertions than deletions [18]. The authors concluded that loops on the primer strand were repaired differently from loops on the template strand.

The role of MutL proteins in MMR is less well understood, although they act downstream of initial mismatch detection [7–9]. In both yeast and mammalian cells, there are three MutL complexes: MutLx, MutLβ, and MutLγ [7–9]. Downstream processing usually involves MutLx, in yeast a heterodimer of Mlh1 and Pms1 [7–9]. In yeast, it appears that both MutLβ (consisting of Mlh1 and Mlh2) and MutLγ (Mlh1 and Mlh3) play a role in correction of deletion mutations, although the effect is minor and depends on a sensitive assay [20,21]. Although MutL proteins are not thought to have any specific recognition of mismatches, two mutations in PMS1, pms1-G882E and pms1-H888R, were shown to result in substantial increases in +1 insertions but had essentially no effect on repair of base-base mismatches or deletions [22].

Biochemical analysis has given no information about how MMR could differentiate between mismatches that would lead to
DNA mismatch repair is a major pathway that prevents both base substitution and insertion or deletion errors during replication. Most eukaryotes have two recognition complexes, MutSα and MutSβ, homologues of prokaryotic MutS and differing in their affinity for mismatches, with MutSα recognizing base-base mismatches and small insertion/deletion loops and MutSβ recognizing larger loops. We show that repair mediated by these complexes has opposite biases for insertion versus deletion mispairs with MutSα-directed repair favoring insertion loops and MutSβ-directed repair favoring deletion loops. This bias is mediated by differing interactions with downstream MutL complexes. We suggest that MutSα represents a prokaryotic MutS biased for repair of insertion loops and that MutSβ represents a new eukaryotic activity biased for repair of deletion loops.

Results

An assay for in/del mutations

We had previously used oligo transformation to study insertion mutations using the *lys2Δ1746* frameshift reversion assay that requires restoration of a −1 frameshift in a region of the *LYS2* gene indifferent to amino acid sequence [23,24]. We wanted to study the effects of MMR on deletion mutations as well as insertion mutations, but, because of the different affinities in binding of loop sizes by MutSα and MutSβ, it was necessary to compare the effects of insertion and deletion mismatches of the same size, requiring the use of two complementary reversion assays. We therefore used both the −1 *lys2Δ1746* frameshift allele and the +1 frameshift allele *lys2ΔBgl* in the same *LYS2* region [23–25]. In order to have reversion windows with known orientations relative to a dependable origin of replication and to have the different frameshift alleles as similar as possible, we used the *LYS2* genes inserted in both orientations (“same” and “opposite”) at the *HIS4* locus previously described [26] and inserted the frameshift alleles as described in MATERIALS AND METHODS. The −1 *lys2Δ1746* frameshift allele was used to study +1 and −2 loops, and the +1 frameshift allele *lys2ΔBgl* was used to study −1 and +2 loops. The overall scheme for the assay is illustrated in Figure 1.

The efficiency of recognition by MMR is known to be dependent not only on the mispaired bases, but also on the sequence context surrounding the mispaired bases [10,27]. Therefore we used a collection of oligos that created different mismatches, in two sequence contexts (Figure 1B). Because we transform with single-stranded oligos, the oligos can have the sequence of the transcribed strand and create a TC or GA insertion loop or have the sequence of the nontranscribed strand and create a TC or GA insertion loop, all in otherwise the same sequence context. This was done in two different locations within the reversion windows of the *lys2* mutant alleles. Deletion loops are created by transforming with oligos lacking certain bases contained in the template strand; therefore different deletion loop sequences cannot be created in the same sequence context using the same strains.

Oligos induce 2-nt insertion or deletion mutations with approximately the same efficiency in the absence of MMR

As detailed in Materials and Methods, an oligo was transformed into a given strain in three independent experiments, and the average number of transformants over background reversion events was determined. All oligos were transformed into two strains with opposite orientations of the *LYS2* gene relative to the nearby origin of replication so that the effect of loops on the leading versus lagging strand could be assessed. The results of transformation with a selected set of oligos in strains containing or lacking certain components of MMR are given in Figure 2 and the full set of results is given in Figure S1.

Several patterns can be observed in the results in strains lacking MMR (*msh2*). Each pair of oligos differing only in the insertion bases gave results that were generally not statistically different from one another. Comparing any set of oligos (e.g. TrL1-Lag-s), the difference between insertions and deletions was generally less than 2-fold, with a mixture of insertions, deletions, or neither predominating. Finally, as we have observed previously [23,26], in all cases the number of insertions or deletions was greater when targeted to the lagging strand than to the leading strand, by an average of approximately 6-fold in these experiments. Therefore we can conclude that, in the absence of MMR, insertion and deletion mutations can be created at approximately equal efficiencies by oligos.

MutSα and MutSβ have opposite effects on 2-nt insertion versus deletion mismairs

In contrast to oligo transformation in the absence of MMR, one can see quite different patterns of transformation in strains containing only MutSα (*msh3* strains) or MutSβ (*msh6* strains) in Figure 2. To compare the effect of MMR on transformation, we divided the average number of revertants obtained in the absence
Figure 1. An assay for loop repair. (A) The initial strains used to construct the assay for in/del loop repair were a set of isogenic strains containing the LYS2 gene replacing the HIS4 gene near the ARS306 origin of replication as shown above [26]. “Same” and “Opposite” refer to the orientation of the LYS2 gene relative to the orientation of the original HIS4 gene [26]. The wild-type LYS2 sequences were subsequently replaced with sequences to create either the −1 frameshift allele lys2ΔA746 or the +1 frameshift allele lys2ΔBgl [23–25]. (B) The −1 lys2ΔA746 and the +1 lys2ΔBgl frameshift alleles can be reverted to wild-type by a compensating addition or deletion of nucleotides anywhere within an approximately 200-bp reversion window [23–25]. Oligos with sequences corresponding to two different locations within the reversion window of the mutant alleles and ranging in size from 31–36 nt were used to produce Lys+ revertants (Table S4). The colors indicated are those used in subsequent figures and also in Table S4. The red and yellow oligos induce a 2-nt loss in lys2ΔA746 strains and the blue and green oligos insert 2 nt into lys2ΔBgl strains. Single-stranded oligos are used for transformation, and can therefore have the sequence of either the transcribed (Tr) or non-transcribed strand (NTr). Oligos inducing 1-nt in/del mutations follow a similar color and naming scheme (see text for details). (C–F) Oligos transform by serving as primers for subsequent replication, on either the leading or lagging strands of replication. If the mismatch created by the oligo is not removed during replication, a reverting frameshift will result in the next round of replication. Additional nucleotides in the oligo will create a primer-strand loop and thus an insertion mutation; missing nucleotides in the oligo will create a loop on the template strand and thus lead to a deletion mutation. (C) and (D) indicate that the same oligo (an oligo with the sequence of the transcribed strand (Tr) in location 2 (L2) adding a sequence of TC) will anneal to the leading strand of replication in lys2ΔBgl strains of the Opposite orientation (TrL2-lead-o) or to the lagging strand in strains of the Same orientation (TrL2-lag-s). (E) and (F) show the same process for an oligo inducing a deletion of GA in lys2ΔA746 strains by annealing on the leading strand in strains of the Opposite orientation (TrL2-lead-o) or on the lagging strand of strains in the Same orientation (TrL2-lag-s).

doi:10.1371/journal.pgen.1003920.g001
Mismatch Repair of In/Del Loops

of MMR by the average number of revertants obtained in a given MMR background to give a Repair Ratio (Table 1). The larger the Repair Ratio, the more effectively the loop created by the oligo was removed. The results in strains containing only MutSβ (msh6 strains) are very consistent, as can be seen in Figures 2 and S1 and Table 1. In every case, deletion mispairs were corrected much more efficiently than insertion mispairs; in Table 1, the Repair Ratios for insertions range from 1 to 13 and for deletions from 59 to 310. The results in strains containing only MutSa (msh3 strains) were more varied. Uniformly, deletion mispairs are poorly repaired, with a range of Repair Ratios from 2 to 9 (Table 1). Insertion mispairs are repaired with a wide range of efficiencies of 2 to 130 (Table 1). The one consistent difference is that within the same sequence context, a GA sequence in the loop is always repaired more efficiently than a TC. However, when only MutSa is present, insertion loops are repaired overall with much greater efficiency than deletion loops. Additionally, in the presence of only MutSa, insertion loops are repaired with somewhat greater efficiency when the loop is on the lagging strand compared to the leading strand, with an average ratio of 1.6, whereas, when only MutSβ is present, deletion loops on the leading strand are repaired 1.6-fold more efficiently than on the lagging strand, a difference we previously found under other circumstances [23]. The difference between these two ratios is statistically significant as determined by a Mann-Whitney rank sum test (P = 0.038). A median measure of the insertion and deletion loop Repair Ratios is given in Table 2, which illustrates the differing biases of MutSa and MutSβ.

Deletion mispairs of 2 nt are more efficiently corrected than insertion mispairs in wild-type strains

There is inherently more error associated with measurement of revertants in cells that are wild type for MMR, as the number of revertants can be decreased by over two orders of magnitude to quite low numbers. However, a consistent pattern emerges as observed both in Figures 2 and S1 and in Tables 1 and 2: 2-nt deletion mispairs are corrected more efficiently than insertion mispairs in strains wild type for MMR. Deletion mispairs are corrected with an efficiency somewhat greater than that of cells containing MutSβ alone (usually less than two-fold), presumably reflecting the ability of MutSβ to recognize deletion mispairs, albeit at a much lower efficiency than does MutSβ. Insertion mispairs are generally corrected with an efficiency greater than that observed in cells with MutSβ alone, although in 5 cases, insertion mispairs were corrected less efficiently than in MutSa cells, and in one other case about the same (Table 1). One explanation for those situations could be a dilution in MutSa molecules due to Msh3 pairing with some of the Msh2 [13,14]. A dinucleotide repeat stability assay previously showed that 2-nt deletions were repaired with a greater efficiency than insertions in strains wild-type for MMR [18,19].

Figure 2. Effect of MMR on 2-nt in/del mismatches. The mean number of Lys+ revertants, with standard deviation, is shown for the indicated oligo and strain combination. The coloring is explained in Figure 1 and oligo sequences are given in Table S4. TrL1 and TrL2 refer to oligos with the sequence of the transcribed strand in Location 1 and 2, respectively. For the Tr oligos, annealing to the lagging strand occurs in strains with the Same orientation (Lag-s). The fewer transformants obtained for a given oligo and strain combination, the better the repair for the mismatch created by the oligo. Oligos creating insertion loops are transformed into lys2ΔBglI strains and oligos creating deletion loops are transformed into lys2ΔA746 strains. As an example, all TrL1 oligos are essentially identical in sequence, with the exception that the “blue” oligo inserts a +GA loop, the “green” oligo inserts a +TC loop, and the “red” oligo causes a 2-nt –GA deletion loop in the template strand opposite the location of the + loops in the other two oligos. There is no active MMR in msh2 strains, whereas msh3 strains have MutSα present and msh6 strains contain MutSβ.

doi:10.1371/journal.pgen.1003920.g002
Two mutations in *PMS1*, *pms1-G882E* and *pms1-H888R*, result in repair deficiency of 2-nt insertions

A screen for mutations in *PMS1* found two mutants that resulted in large increases in +1 insertions but had no effect on deletions [22]. We tested those mutations in our assay system to see if they would have a similar effect on 2-nt in/del mispairs. The results are shown in Figures 3 and S2.

As described in Materials and Methods, the *pms1(761-904)*A mutant was a precursor in construction of the two *PMS1* point mutations; terminal deletions of that length have previously been shown to be nonfunctional [29]. Pms1 is needed for most repair, as the *pms1(761-904)*A strains behave similarly to the *msh2* strains. However, the *msh2* strains generally had more transformants, averaging 1.7-fold more insertions and 2.5-fold more deletions (Table S1), suggesting that some in/del repair might be mediated by complexes lacking Pms1. Strains containing either of the two *PMS1* point mutations show an extreme difference in repair of insertion versus deletion mispairs that is evident in Figures 3 and S2 and given quantitatively in Tables 1 and S1. Both mutant strains repair deletion mispairs but have little effect on insertion mispairs. The median effect of each mutation is presented in Table 2. The effect of the two insertions, *pms1-G882E* and *pms1-H888R* are similar, but the *pms1-H888R* mutants appear to have a more distinctive effect, with almost no repair of insertion mispairs but more repair of deletion mispairs than the *pms1-G882E* mutants. Because there is very little repair of deletion mispairs in the absence of Pms1 (Table S1), the *pms1* point mutants must be functional in deletion repair.

### Similar MMR effects are observed in 1-nt in/del mispairs

Previously, the evidence for the differential effect of MutSα and MutSβ on in/del mutations came from a dinucleotide repeat assay, although an assay using one particular mononucleotide repeat indicated that the loss of either MutSα or MutSβ led to an increase mainly of deletions [18]. The *pms1-G882E* and *pms1-H888R* mutations had only been examined with mononucleotide repeats [22]. Therefore we wanted to examine whether the effects we observed on 2-nt in/del mispairs would be observed in similar 1-nt in/del mismatches. For that survey, we used only oligos in one location, and the results are presented in Figures 4 and S3; quantitative comparisons are given in Table 3.

There are similarities to the results with 2-nt in/del mismatches in terms of the opposing biases for insertions versus deletions, but the quantitative results differ, presumably due to the relatively greater affinity of MutSα recognition for 1-nt loops over 2-nt loops, and the correspondingly lower recognition of MutSβ for 1-nt loops compared to 2-nt loops. MutSα has an overall much greater effect on suppression of 1-nt in/del mismatches than does MutSβ, and MutSβ has substantial activity on 1-nt deletion loops in contrast to its activity on 2-nt deletion loops (Figures 4 and S3). Even so, MutSα has a consistently greater activity toward 1-nt insertion mismatches, whereas the MutSβ activity is the reverse. In contrast, the *pms1-G882E* and *pms1-H888R* mutants have about the same lack of insertion repair as exhibited on 2-nt in/del mispairs (Tables 3, S2). However, deletion repair in the *pms1-G882E* and *pms1-H888R* mutants is much more efficient than that in strains containing only MutSβ, indicating the involvement of MutSβ in 1-nt deletion loop repair. The median Repair Ratios are

### Table 1. Repair Ratios for 2-nt in/del mispairs.

| Location 1 | Tr | NTr | wt | msh3 | msh6 | pms1-H888R | pms1-H888R msh3 |
|------------|----|-----|----|------|------|------------|----------------|
| Lag-s      | +GA | 200 | 130| 13   | 1.1  |            |                |
| Lag-o      | +TC | 18  | 5  | 4    | 1.1  |            |                |
| Lag-s      | +TC | 95  | 55 | 5    | 1.0  |            |                |
| Lag-o      | +GA | 58  | 29 | 8    | 1.5  |            |                |
| Lead-o     | +GA | 58  | 55 | 6    | 1.3  |            |                |
| Lead-s     | +TC | 24  | 4  | 8    | 1.1  |            |                |
| Lead-o     | +TC | 32  | 22 | 3    | 2.0  |            |                |
| Lead-s     | +GA | 58  | 20 | 12   | 1.4  |            |                |

### Table 2. Median Repair Ratios for 2-nt in/del mismatches.

| MMR Genotype | Insertions | Deletions | Difference* |
|--------------|------------|-----------|-------------|
| msh3 (MutSα) | 26         | 5.5       | P = 0.018   |
| msh6 (MutSβ) | 4.5        | 100       | P < 0.001   |
| wt           | 28         | 220       | P < 0.001   |
| *pms1(761-904)*A | 1.8 | 2.4 | N.S. |
| *pms1-G882E* | 1.9        | 50        | P < 0.001   |
| *pms1-H888R* | 1.2        | 100       | P < 0.001   |
| mlh3         | 180        | 54        | P = 0.008   |
| *pms1-G882E* msh6 | 42       |            |             |
| *pms1-H888R* msh6 | 95       |            |             |
| *pms1-H888R* mlh3 | 16       |            |             |
| *pms1-H888R* msh6 mlh3 | 16       |            |             |

The median Repair Ratio for each genotype is calculated from the values with individual oligos in Tables 1, 5, and S1. *The probability that the values for insertions were different from deletions was calculated using a Mann–Whitney rank sum test. N.S. indicates the two sets of values were not significantly different.

**Table 1.** Repair Ratios for 2-nt in/del mispairs.

**Table 2.** Median Repair Ratios for 2-nt in/del mismatches.
Figure 3. The effect of mutations in PMS1 on 2-nt in/del mispairs. Oligos were transformed into strains of the indicated genotypes and analyzed as in Figure 2; the msh2 results are those given in Figure 2.
doi:10.1371/journal.pgen.1003920.g003

Figure 4. Effect of MMR on 1-nt in/del mismatches. TrL1 Oligos were transformed into Same-orientation strains of the indicated genotypes and analyzed as in Figure 2 (TrL1-Lag-s). For 1-nt in/del mismatches, oligos creating insertion loops are transformed into lys2ΔA746 strains and oligos creating deletion loops are transformed into lys2ΔBgl strains. Only MutSb is present in msh6 strains and only MutSα is present in msh3 strains.
doi:10.1371/journal.pgen.1003920.g004
given in Table 4 and illustrate that in contrast to the situation with 2-nt loops, there is relatively more repair of deletions with MutSx only and relatively less repair of deletions with MutSr only, and in wild-type cells insertions and deletion mismatches are corrected with indistinguishable efficiency.

The interaction of MutSx and PMS1 mutations in in/del repair
How can the specificity of the pms1-G882E and pms1-H888R mutations best be understood? The pms1-G882E and pms1-H888R mutant strains appeared to be similar to msh6 strains lacking MutSx for 2-nt deletion repair; we therefore examined strains containing both msh6 deletions and pms1 mutations to determine if they appeared to be in the same pathway. Because the pms1 mutants fail to repair insertion loops, we could only examine the effect on deletion loop mispairs. The results are given in Figures 5 and S4 and Tables 1, 2, and S1 for 2-nt deletions. Results in the

| Location 1 | Lag-s | −T | 300 | 85 | 24 | 63 | 220 | 38 | 4 | 4 |
|------------|-------|----|-----|----|----|----|-----|----|---|---|
| Lag-o      | −A    | 290| 60  | 22 | 400| 80 | 32  | 29 | 21 |
| Lead-o     | +T    | 120| 210 | 4  | 112| 970|
| Lead-s     | +T    | 210| 170 | 2  | 4  | 730|

Data from Figure S3 were used to calculate Repair Ratios by using the ratio of revertants obtained in the absence of MMR (msh2 strains) with the number of revertants in strains of the indicated genotype.

doi:10.1371/journal.pgen.1003920.t003

For 1-nt deletion loop repair, MutSx is much more important than in 2-nt loop repair and as noted above, the repair in the pms1 mutants is more efficient than in the presence of only MutSb. Repair of 1-nt deletion loops in the double pms1-G882E msh6 and pms1-H888R msh6 mutants is much lower than in the single pms1 mutants (Figure 4; Tables 3, S2), indicating that much of the deletion loop repair in the pms1 mutants must be due to the action of MutSx (Table 4).

The role of MutLγ in in/del repair
In order to determine if MutLγ (composed of Mlh1 and Mlh3 subunits [21]) might be involved in some of the observed repair, we examined strains with an Mlh3γ deletion. The results for 2-nt in/del mispairs are given in Figures 5 and S4 and Tables 2 and 5. It is evident that MutLγ is not involved in repair of insertion mispairs, as Repair Ratios actually increased in the absence of MutLγ (P = 0.019) (perhaps due to a somewhat increased amount of MutLx). An mlh3γ deletion resulted in an approximately 4-fold decrease in repair of deletion mispairs (Table 2) (P = <0.001). Those results were expected given the limited effect previously found for msh3 deletions [10,20,21]. The pms1-H888R mutation has less than a 2-fold effect on deletion repair, so one would have anticipated that the double mutant would be similar to the mih3 mutant. Such was not the case as seen in Tables 2 and 5. The double mutant had an almost 13-fold reduction in deletion repair compared to wild type. The difference between repair in msh3 and pms1-H888R mih3 strains is significant, with P = <0.001.

The same pattern was found in 1-nt in/del mismatch repair. A single mih3 deletion has a relatively small effect on in/del repair, slightly raising the efficiency of insertion repair compared to wild type and slightly decreasing the efficiency of deletion repair, although the difference in both cases is marginally significant (P = 0.05) (Tables 3 and 4). The pms1-H888R mutant has robust deletion repair, but the double mutant pms1-H888R mih3 was reduced by 20 fold in deletion repair (the difference is significant, with P = 0.029); deleting msh6 had no further effect (Tables 3 and 4). This result was particularly surprising, as MutSx is responsible for much of the 1-nt deletion repair and yet MutLγ has been thought to work only with MutSb [21].

Table 3. In/del Repair Ratios for 1 nt mispairs.

| Location 1 | Lag-s | −T | 300 | 85 | 24 | 63 | 220 | 38 | 4 | 4 |
|------------|-------|----|-----|----|----|----|-----|----|---|---|
| Lag-o      | −A    | 290| 60  | 22 | 400| 80 | 32  | 29 | 21 |
| Lead-o     | +T    | 120| 210 | 4  | 112| 970|
| Lead-s     | +T    | 210| 170 | 2  | 4  | 730|

The median Repair Ratio for each genotype is calculated from the values with individual oligos in Tables 3 and S2.

The probability that the values for insertions were different from deletions was calculated using a Mann-Whitney rank sum test. N.S. indicates the two sets of values were not significantly different.
doi:10.1371/journal.pgen.1003920.0004

Table 4. Median Repair Ratios for 1 nt in/del mismatches.

| MMR Genotype | Median Repair Ratio |
|--------------|---------------------|
| msh3 (MutSx) | 190                 |
| msh6 (MutSr) | 3                   |
| wt           | 220                 |
| pms1-G882E   | 2.3                 |
| pms1-H888R   | 2.3                 |
| mih3         | 850                 |
| msh6         | 15                  |
| pms1-H888R msh6 | 15             |
| pms1-H888R mih3 | 20              |
| pms1-H888R mih3 | 15              |

The median Repair Ratio for each genotype is calculated from the values with individual oligos in Tables 3 and S2.

The probability that the values for insertions were different from deletions was calculated using a Mann-Whitney rank sum test. N.S. indicates the two sets of values were not significantly different.
doi:10.1371/journal.pgen.1003920.0004
Discussion

The biases we find here for repair of in/del mispairs had been previously observed in two different systems: a dinucleotide repeat assay for MutSα and MutSβ [18,19] and frameshift reversion assays for the pms1 mutants [22]. Given the limited scope of each of those experiments, it was not clear whether the results reflected a general property of the proteins involved, or were influenced by the DNA sequences involved in the particular assays used. Our results with a completely different assay system and with a variety of different sequences and gene strands and orientations lend confidence that our observations reflect an inherent difference in repair of insertion versus deletion loops by MMR.

For 2-nt in/del mismatches, strains containing only MutSβ provide the clearest picture of a bias. As shown in Table 1, the repair of all insertion loops tested is poor, ranging from 1 to 13-fold, and the repair of all tested deletion loops is robust, ranging from 60 to 300-fold. Although MutSβ has a measurable effect on repair of most insertions, it is only deletions for which it has a substantial effect. The effect in strains containing only MutSα is a bit more complex. The repair of deletion loops is uniformly low, ranging from 2 to 9-fold (Table 1). The repair of insertion loops is

Table 5. The effect of Mlh3 on Repair Ratios for 2-nt in/del mispairs.

| Location 1 | Tr | NTr | mutl3 | Tr | NTr | mutl3 | pms1-H888R mutl3 | pms1-H888R msh6 mutl3 |
|------------|----|-----|-------|----|-----|-------|-----------------|---------------------|
| Lag-s      | +TC| 280 | -GA   | 87 | 14  | 14    |                 |                     |
| Lag-o      | +GA| 160 | -TC   | 56 | 18  | 21    |                 |                     |
| Lead-o     | +TC| 85  | -GA   | 68 | 14  | 17    |                 |                     |
| Lead-s     | +GA| 210 | -TC   | 78 | 19  | 13    |                 |                     |
| Location 2 |    |     |       |    |     |       |                 |                     |
| Lag-s      | -  | -   | -     | -  | -   | TC    | 32              | 12                  |
| Lag-o      | -  | -   | -     | -  | -   | GA    | 51              | 18                  |
| Lead-o     | -  | -   | -     | -  | -   | TC    | 23              | 13                  |
| Lead-s     | -  | -   | -     | -  | -   | GA    | 51              | 24                  |

Repair Ratios were calculated as in previous tables.

doi:10.1371/journal.pgen.1003920.t005
It is not clear how the two \textit{pms1} mutants affect MMR. The two \textit{pms1} mutations map into a region described as an \textit{Mlh1}-interaction region [29], but the interaction of the mutant proteins with \textit{Mlh1} was found not to be defective as judged by a two-hybrid assay [22]. A recent structure of the \textit{S. cerevisiae} \textit{MutL\alpha} C-terminal domain permits a much better understanding of the location of the mutations within the \textit{MutL\alpha} protein [32]. At the time of the Erdenz\i et al. paper, the initiating ATG codon was thought to be in a location such that the length of the \textit{Pms1} protein would be 904 aa. However, a genomic analysis found a different ATG codon to be the correct initiation site for translation, leading to a predicted protein length of 875 aa [33]. With that numbering, the two \textit{Pms1} mutations would be G851E and H857R. The crystal structure shows that the H857 residue is centered in the \(\beta\) \(\beta\)-sheet that is part of one of the most important regions of the heterodimerization interface, Patch 1 [32]. The G851 residue sits just outside the \(\beta\) \(\beta\)-sheet and so it is reasonable to suppose that a mutation in that residue could affect \textit{Pms1}-\textit{Mlh1} interaction. One of the zinc atoms in the endonuclease site is stabilized by C848 and H850 [32]. That would put the G851 residue close to the endonuclease site, making it possible that the G851E substitution might interfere with the binding of the zinc atom and thus affect endonuclease activity. However, there is no indication in the structure that the H857 residue would influence endonuclease activity, and as we found above, the H857R mutation has a more distinctive mutator effect than the G851E mutation. Both of the mutations were found to have essentially wild-type base-base MMR activity [22], and as \textit{Pms1} endonuclease activity is crucial for MMR function [34], we consider it highly unlikely that the effects of the two mutations is on the endonuclease activity of \textit{Pms1}.

In accordance with previous results, we find that the absence of \textit{Mlh3} leads to somewhat less effective repair of deletion mispairs (Tables 2 and 4) [20,21]. The repair of insertion mispairs in an \textit{mlh3} background is more robust than in a wild-type background, suggesting that the loss of \textit{Mlh3} might lead to a somewhat greater amount of \textit{MutL\alpha} in the cell, with correspondingly greater repair of insertions. That view is consistent with the previous observation that overexpression of \textit{Mlh3} appears to result in lower levels of \textit{MutL\alpha} [35]. The surprise was the deletion repair observed in \textit{pms1-H858R} \textit{mlh3} mutants. Given the small effect of each individual mutation on deletion repair, one would have expected deletion repair to be robust in that mutant background. Instead, repair of both 1-nt and 2-nt deletion mispairs was synergistically compromised (Tables 2 and 4). Based on the prior results with the \textit{pms1-H808R} mutants, it appeared that only insertion repair was compromised [22]. Our results suggest a different possibility: although the \textit{pms1-H808R} mutant is functional for base mismatch repair, it functions relatively poorly in \textit{in/del} mismatch repair. One possible explanation for this hypothesis involves the finding that \textit{MutS\alpha} complexes recognizing mismatches are responsible for loading multiple copies of \textit{MutL\alpha} onto DNA [36]. \textit{MutS\alpha} recognizing a base-base mispair can interact with the \textit{pms1-H808R} mutant to create a functional complex. However because of the orientation of the proteins mediated by their binding to PCNA, neither \textit{MutS\alpha} nor \textit{MutS\beta} when recognizing an insertion mispair can interact properly with the \textit{pms1-H808R} mutant complex and there is very little insertion repair. When \textit{MutS\beta} recognizes a deletion mispair, the complex is positioned so that it is able to interact with the \textit{pms1-H808R} mutant \textit{MutL\alpha}, although relatively poorly, giving Repair Ratios of 16–20 (Tables 2 and 4). This interaction is facilitated by \textit{MutL\alpha} interacting with \textit{MutS\beta}, which then helps recruit multiple molecules of the \textit{pms1-H808R} mutant complex.
Repair of 2-nt deletion loops by MutS\(\alpha\) is poor (Repair Ratio of 5.5, Table 2); however repair of 1-nt deletion loops by MutS\(\alpha\) is much more robust (Repair Ratio of 73, Table 4), although still less than insertion loop repair. Repair of 1-nt deletion loops in the \textit{pms1-H888R} mutant is much greater than repair with only MutS\(\beta\) present (Repair Ratio of 170 compared to 22, Table 4), suggesting that much of the repair in the \textit{pms1-H888R} mutant must be by MutS\(\alpha\). The fact that repair of 1-nt deletion loops in the \textit{pms1-H888R mlh3} background drops to the level of repair when only MutS\(\beta\) is present suggests that MutS\(\alpha\)-directed repair in the presence of the \textit{pms1-H888R} mutation involves MutL\(\gamma\). The very modest effect of the \textit{mlh3} mutation by itself shows that normal MutS\(\alpha\)-directed repair of 1-nt deletion loops does not use MutL\(\gamma\); confirmation of this suggestion would require additional experiments. One issue that has not been clear from previous experiments because of the modest effect of MutL\(\gamma\) on repair is whether there were certain mismatches that required MutL\(\gamma\) function, perhaps instead of MutL\(\alpha\), or whether the action of MutL\(\gamma\) always required MutL\(\alpha\) and any mismatch was potentially susceptible to MutL\(\gamma\) function. Because each of our assays examines only one particular mismatch and because we see a strong effect in the \textit{mlh3 pms1-H888R} background, we can draw several conclusions. 1) MutL\(\gamma\) functions only in repair of deletion loops and not insertion loops. 2) Any deletion loop is susceptible to being aided in repair by MutL\(\gamma\). 3) MutL\(\gamma\)-mediated repair also requires MutL\(\alpha\). These conclusions do not mean that the effect of MutL\(\gamma\) deletion would be the same for all deletion loops; for both 1-nt and 2-nt deletion loops there is a range of about 4-fold in Repair Ratios, suggesting that certain mismatches could be more dependent for MutL\(\gamma\) on their repair.

The above model, while compatible with our results, makes several predictions that may however prove difficult to study. The first is that the bias in repair of insertions compared to deletions is ultimately a function of the MutL complexes and not the recognition by MutS complexes. A role for MutL\(\gamma\) in the repair of some deletion mispairs had previously been detected [20,21], so the idea that MutL complexes could be biased in in/del repair is not without precedent. Secondly, the bias observed in in/del repair mediated by MutS\(\alpha\) and MutS\(\beta\) indicate that they contact MutL\(\alpha\) differently such that a deletion mispair recognized by MutS\(\beta\) is more likely to be repaired than if the same mispair were recognized by MutS\(\alpha\), and vice versa for insertion mispairs. A major question then is how the MutS and particularly the MutL components could be oriented such that an insertion mispair was recognized differently from a deletion mispair.

An important part of the explanation likely involves interactions of MMR proteins with the proliferating cell nuclear antigen, PCNA. PCNA is a family of DNA sliding clamps that encircles DNA, is essential for replication, and has binding sites for many proteins, including the replicative polymerases [37] and there is evidence that it can act as a scaffold to coordinate MMR through consecutive protein-protein interactions [38]. PCNA is required for MMR at a step preceding DNA resynthesis [39,40], and MMR interactions with PCNA could be responsible for strand discrimination [41,42]. A variety of experiments demonstrated direct interactions of PCNA with Mlh1, Msh3, and Msh6, and those interactions were important for proper MMR [40,43–46]. It is clear that interaction with PCNA is not sufficient to drive MMR, as there are other processes occurring. For example, engineering a mutation that blocked MutS\(\alpha\) conformational change upon mismatch binding demonstrated that such change was necessary for MutL\(\alpha\) binding [47]. PCNA is asymmetrical with respect to the replication fork, and this asymmetry can result in specific MutL\(\alpha\) loading and subsequent endonucleolytic activation and thus proper strand discrimination as has been observed in human MMR [42]. Importantly for this work, experiments with various PCNA mutants suggested that the interactions of PCNA are different for Msh3 compared to Msh6 [48]. In addition, it has been recently shown in humans that in contrast with MutS\(\alpha\), PCNA and MutL\(\alpha\) have the same binding site on MutS\(\beta\), suggesting that the interaction of MutS\(\beta\) with PCNA and MutL\(\alpha\) would be sequential [49]. These considerations suggest a mechanism by which the recognition of, for example, an insertion loop could be different for MutS\(\alpha\) compared to MutS\(\beta\) because of their different orientation to the duplex bulge due to their different PCNA interaction. It is not clear how subsequent interactions with MutL complexes are handled. In vitro studies suggest that MutS\(\alpha\) is bound to PCNA on homoduplex DNA, and, when a mispair is encountered, the interaction with PCNA is either lost or changed [50]. The next step of interaction with MutL complexes could be sequential for both MutS\(\alpha\) complexes, with a loss of the MutS interactions [38], but given the different nature of the MutS complex interactions with PCNA [49], the nature of the interactions of MutS\(\alpha\) and MutS\(\beta\) with MutL\(\alpha\) is likely to be very different.

It is surprising to find that insertion and deletion mispairs are repaired with differing biases and that MutS\(\alpha\) and MutS\(\beta\) exhibit opposite biases for such repair. What might account for the development of an MMR system that would function in such a manner? A recent analysis was done of multiple strains of over 40 bacterial and archaeal species. It was found that in species with no MMR system, expansions and contractions of simple sequence repeats were equally likely, whereas in species containing MMR systems, there was a bias toward contraction of simple sequence repeats [51]. Thus, it appears that bacterial and archael MMR systems, like yeast strains containing only MutS\(\alpha\), repair insertions better than deletions. It is possible that such a bias could have an evolutionary advantage, tending to reduce the length of simple sequence repeats. Although most eukaryotic species seem to have an MMR system, not all have a MutS\(\beta\); in fact two favorite model organisms, \textit{D. melanogaster} and \textit{C. elegans}, lack MutS\(\beta\), although they both have MutS\(\alpha\) [17]. Structural evidence also shows that MutS\(\alpha\) binds mismatches in a manner similar to MutS\(\alpha\), whereas MutS\(\beta\) binds mismatches quite differently [30]. This analysis would suggest that MutS\(\alpha\) represents the bacterial MutS activity, whereas MutS\(\beta\) represents a new activity in which the bias toward repair of deletion mispairs may have been equally or more important than the recognition of larger loops. Many eukaryotic organisms have abundant simple sequence repeats, including those in exons, and the addition of a more robust activity repairing potential deletion mispairs would help preserve those repeats in the genome. This new MutS\(\beta\) activity, due to the \textit{MSE3} gene, not only had a recognition specificity different from that of MutS\(\alpha\), but interacted in a somewhat different manner with PCNA and MutL\(\alpha\) and the new MutL\(\gamma\) complex that apparently does not usually interact with MutS\(\alpha\) [21]. Domain swap experiments have shown that the mismatch recognition domain of Msh3 is not necessary for interaction with MutL\(\gamma\), but rather another part of the Msh3 protein present in MutS\(\beta\) [52].

Given the high degree of conservation, in both sequence and function, between MMR systems in yeast and mammalian cells, our results likely apply also to mammalian cells, although the experiments to test that are much more difficult to carry out. Repeat stability is a concern for mammalian cells, both in terms of various trinucleotide repeat diseases and in cancer [53,54]. In various trinucleotide repeat diseases, there is a strong involvement with MMR, but the effects are complicated [53]. In a mouse model of Friedrich ataxia which has GAA repeats, repeat
instability was increased in the absence of MMR and there were enhanced deletions in the absence of MutSβ and an enhancement of both deletions and insertions in the absence of MutSα, with a relatively greater increase in insertions [55]. Those results are consistent with the activities we report here. However, repeat instability of other types of trinucleotide repeats shows a different effect, with MMR appearing to be required for expansion, for example [53]. Although there is not a complete understanding of such effects, many of them involve MutSβ and interactions with larger loops. For example, there are certain types of loops that are repairable by MutSβ and others such as CAG loops in which the loop appears to maintain MutSβ binding, thus preventing repair [56]. However, in an in vitro assay, 1 or 2 repetitions of CTG/CAG were repaired in a process requiring MutSβ, but not larger loops, or substrates that contained multiple loops on both strands [57].

Some of the first analyses of MMR genes in humans demonstrated that defects in MMR led to Lynch syndrome or hereditary nonpolyposis colorectal cancer and that such cells manifested a greatly enhanced microsatellite instability [1,2]. Although the overall mutator effect of deficiencies in MMR is likely important in tumor formation and progression, genes containing exonic microsatellite sequences are a particularly susceptible target as any alteration in such sequences will likely lead to a strong phenotype [54,58,59]. Additionally there is some evidence that microsatellite repeats within introns and in 5′ and 3′ untranslated regions could also contribute to carcinogenesis [54]. Not only is the distribution of different tumor types generally different in MMR-defective mice compared to humans, but there is a marked difference depending on the particular defect in MMR [54,60]. Our results provide additional information on possible reasons for those differences. Part of the difference between the distribution of tumor types in mouse and human is likely due to the difference in the existence and sequence of regions in cancer target genes susceptible to in/del formation. Although we are able to induce approximately equal frequencies of insertion or deletion mispairs in the absence of MMR, spontaneous formation of primer or template loops could be at least partially a function of sequence, sequence context, and replication on the leading versus lagging strand, thus also implicating the relation of the gene to replication origin. Because there is plasticity in use of replication origins, the same gene could be replicated differently depending on tissue type [61]. Not only could the formation of a loop be influenced by its sequence and location near an origin, but as we have demonstrated previously [23] and also find here, there is a bias in repair by MutSα and MutSβ depending on the replication strand. There is some variability with MutSβ with different oligos, but there is even more pronounced variability with MutSα, with almost a 100-fold difference in repair between the best- and worst-repaired oligo (Table 1). In both yeast and human cells, there seems to be generally more MutSα than MutSβ in cells, so the likelihood of repair of a given in/del will depend on how well it is recognized by MutSα or MutSβ, which could depend on a variety of factors including sequence and perhaps location, whether it is an insertion or deletion loop, and on which replication strand it appears on. If there turns out to be significant variability in the relative amounts of MutSα and MutSβ in various tissues, as has been found in mouse [16], the likelihood of repair could depend on tissue type. We demonstrate here the surprising finding that although the recognition of in/del mispairs is due to the MutS complex, it is the interaction with the MutL complex that biases the efficiency of repair of an insertion versus deletion mispair. Thus mutations in the genes encoding MutLα could influence not only the efficiency of repair but its bias in repair of in/del mispairs.

### Materials and Methods

**S. cerevisiae strains and oligos**

The genotypes of strains used in these experiments can be found in Table S3. All strains were derivatives of SJR2259 and SJR22609 [26] with LLS2 moved into HIS4 location. Mutant yly2 alleles either with [+1] {yly2SAA eradicate} and yly2OABg or [-1] {yly2AAA eradicate} and yly2OA eradicate} frameshifts were then introduced by two-step allele replacement [62] using plasmids pSR125 [63] or pSR786 [64] respectively. ‘S’ and ‘O’ refer to the orientation of the LLS2 gene - the same or opposite orientation relatively to original HIS4 orientation (Figure 1A). Gene deletions were made using a PCR fragment generated from the collection of yeast gene deletions [65]. The pns1 point mutations were made using the delitto perfetto method [66]. The pCORE cassette was inserted into the PMS1 gene using primers GCP735 and GCP736 (Table S4) creating the pns1{761-994}A mutant. The pCORE cassette was then replaced by transformation with a PCR product from strain NEY398 or NEY402 [22] using primers GCP737 and GCP738 (Table S4). Oligos for transformation were gel purified (Eurofins MWG Operon) and are listed in Table S4.

**Transformation with oligos**

Transformation by electroporation was performed essentially as described previously [28,67]. An overnight culture of yeast cells (0.5 ml) was inoculated into 25 ml of YPAD [68], incubated with shaking at 30°C to an A600 of 1.3–1.5, washed twice with cold H2O, and once with cold 1 M sorbitol. After the final centrifugation, all solution was removed from the cells and 150 μl of cold 1 M sorbitol added to resuspend the cells. After addition of 200 pmol oligo and 50 ng of pRS314 [69] plasmid DNA, the solution was mixed and transferred into a 2-mm gap electroporation cuvette and electroporated at 1.55 kV, 200 μF (BTX Harvard Apparatus ECM 630). Immediately after electroporation, the cell suspension was added into 5 ml YPAD to recover for 2 h with shaking at 30°C. Then cells were centrifuged, washed with H2O, and plated on synthetic dextrose (SD) medium lacking lysine [68]. The number of Tpr+ transformants resulting from the pRS314 plasmid served as a useful marker of successful transformations, but was not consistent enough to be used as an internal standard for transformation efficiency. In order to determine background reversion, the same strains were electroporated as described but without adding oligos. For each oligo and strain combination, three independent experiments were performed, and the mean and standard deviation of the number of total transformants calculated.

**Supporting Information**

Figure S1  Effect of MMR on 2-nt in/del mismatches. The mean number of Lys+ revertants, with standard deviation, is shown for each oligo and strain combination. The coloring is explained in Figure 1 and oligo sequences are given in Table S4. TrL1, TrL2, NTrL1, and NTrL2 refer to oligos with the sequence of the transcribed or nontranscribed strand in Location 1 and 2, respectively. For the Tr oligos, annealing to the lagging strand occurs in strains with the Same orientation (Lag-s), and to the leading strand in the Opposite orientation (Lead-o); the reverse is true for NTr oligos. Oligos creating insertion loops are transformed into yly2AB eradicate strains and oligos creating deletion loops are transformed into yly2AAA eradicate strains. As an example, all TrL1 oligos are identical in sequence, with the exception that the...
“blue” oligo inserts a +GA loop, the “green” oligo inserts a +TC loop, and the “red” oligo causes a 2-nt −GA deletion loop in the template strand opposite the location of the + loops in the other two oligos. (TIF)

**Figure S2** The effect of mutations in **PMS1** on 2-nt in/del mispairs. Oligos were transformed into strains of the indicated genotypes and analyzed as in Figure S1; the **msh2** results are those given in Figure S1. (TIF)

**Figure S3** Effect of MMR on 1-nt in/del mismatches. Oligos were transformed into strains of the indicated genotypes and analyzed as in Figure S2. For 1-nt in/del mismatches, oligos creating insertion loops are transformed into **lys2AΔ476** strains and oligos creating deletion loops are transformed into **lys2AΔ6g1** strains. Oligo sequences are given in Table S4. Only MutSβ is present in **msh6** strains and only MutSα is present in **msh3** strains. (TIF)

**Figure S4** Effect of Mlh3 on 2-nt deletion mispairs. Oligos were transformed into strains of the indicated genotypes and analyzed as in Figure S1. (Data for **msh2**, **msh6**, and **pms1-H888R** from Figures S1 and S2.) (TIF)

**Table S1** Repair Ratios for 2 nt in/del mispairs. (DOCX)

**Table S2** Repair Ratios for 1 nt in/del mispairs in **pms1-G882E**. (DOCX)

**Table S3** **S. cerevisiae** strains. (DOCX)

**Table S4** Oligos used in this study. (DOCX)

**Acknowledgments**

We thank Sue Jinke-Robertson, Nayun Kim, and Mike Liskay for plasmids and strains. We thank Natasha Deygayeova, Kirill Lobaches, Francesca Storici, and Rachelle Spell for helpful comments on the manuscript.

**Author Contributions**

Conceived and designed the experiments: GFC. Performed the experiments: NVR. Analyzed the data: NVR. GFC. Wrote the paper: NVR. GFC. Performed the experiments: NVR. GFC.

**References**

1. Fishel R, Leseo MK, Rao MRS, Copeland NG, Jenkins NA, et al. (1993) The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. Cell 75: 1027–1038.

2. Leach FS, Nikolaidis NC, Papadopoulos N, Liu B, Jen J, et al. (1993) Mutations of the wild type **MLH1** gene is a feature of hereditary nonpolyposis colon cancer. Nature 381: 405–409.

3. Marra G, Iaccarino I, Lettieri T, Roscilli G, Delmastro P, et al. (1998) Mismatch repair deficiency associated with overexpression of the **hMLH1** homolog in hereditary colon cancer. Science 263: 1625–1628.

4. Bronner CE, Baker SM, Morrison PT, Warren G, Smith LG, et al. (1994) Mutations in the DNA mismatch repair gene homologue **MLH1** is associated with hereditary non-polyposis colon cancer. Nature 368: 258–261.

5. Li GM (2000) Mechanisms and functions of DNA mismatch repair. Cell Res 10: 88–96.

6. Iyer RR, Plachnik A, Burdett D, Modrich PL (2006) DNA mismatch repair: functions and mechanisms. Chem Rev 106: 302–323.

7. Jiricny J (2006) The multifaceted mismatch-repair system. Nat Rev Mol Cell Biol 7: 333–346.

8. Harrington JM, Kolodner RD (2007) **Saccharomyces cerevisiae** Msh2-Msh3 acts in repair of base-base mispairs. Mol Cell Biol 27: 6546–6554.

9. Strand M, Earley MC, Crouse GF, Petes TD (1995) Mutations in the **PMS1** on 2-nt in/del mispairs. Oligos were transformed into strains of the indicated genotypes and analyzed as in Figure S1; the **msh2** results are those given in Figure S1. (TIF)

10. Harrington JM, Kolodner RD (2007) **Saccharomyces cerevisiae** Msh2-Msh3 acts in repair of base-base mispairs. Mol Cell Biol 27: 6546–6554.

11. New L, Liu K, Crouse GF (1993) The yeast gene **msh2** is a mismatch repair endonuclease. J Biol Chem 268: 1285–1293.

12. Harré BD, Minesinger BK, Jinks-Robertson S (2000) Discrete gene expression and influenced by the direction of DNA replication. DNA Repair (Amst) 9: 759–763.

13. Rodriguez GP, Romanova NV, Bao G, Rouf NC, Kow YW, et al. (2012) MutSβ does not participate in mismatch repair. Nat Struct Mol Biol 19: 466–473.

14. Greene CN, Jinke-Robertson S (1997) Frameshift intermediates in homopolymer runs are removed efficiently by yeast mismatch repair proteins. Mol Cell Biol 17: 2844–2850.

15. Harfe BD, Jinks-Robertson S (1999) Removal of frameshift intermediates by mismatch repair proteins in **Saccharomyces cerevisiae**. Mol Cell Biol 19: 4766–4773.

16. Kim N, Abdulovic AL, Gealy R, Liskay RM (2005) Novel **PMS1** alleles preferentially affect the repair of primer strand loops during DNA replication. Mol Cell Biol 25: 9221–9231.

17. Kow YW, Bao G, Reeves JW, Jinke-Robertson S, Crouse GF (2007) Oligonucleotide transformation of yeast reveals mismatch repair complexes to be differentially active on DNA replication strand. Proc Natl Acad Sci USA 104: 11352–11357.

18. Harfe BD, Jinks-Robertson S (1999) Mapping of frameshift intermediates by mismatch repair proteins in **Saccharomyces cerevisiae**. Mol Cell Biol 19: 72–78.

19. Johnson RE, Kovvai GK, Prakash I, Prakash S (1996) Requirement of the yeast **MSH3** and **MSH6** genes for **MSH2**-dependent genomic stability. J Biol Chem 271: 7285–7290.

20. Marsischky GT, Kolodner RD (1997) Biochemical characterization of the mammalian MutL homologs Mlh2p and Mlh3p in the removal of frameshift intermediates in budding yeast. Curr Biol 10: 145–148.

21. Gorell M, Storici, and Rachelle Spell for helpful comments on the manuscript.

22. Erdeniz N, Dudley S, Gealy R, Jinke-Robertson S, Liskay RM (2005) Novel **PMS1** alleles preferentially affect the repair of primer strand loops during DNA replication. Mol Cell Biol 25: 9221–9231.

23. Kow YW, Bao G, Reeves JW, Jinke-Robertson S, Crouse GF (2007) Oligonucleotide transformation of yeast reveals mismatch repair complexes to be differentially active on DNA replication strand. Proc Natl Acad Sci USA 104: 11352–11357.

24. Harfe BD, Jinks-Robertson S (1999) Removal of frameshift intermediates by mismatch repair proteins in **Saccharomyces cerevisiae**. Mol Cell Biol 19: 4766–4773.

25. Greene CN, Jinke-Robertson S (1997) Frameshift intermediates in homopolymer runs are removed efficiently by yeast mismatch repair proteins. Mol Cell Biol 17: 2844–2850.

26. Kim N, Abdulovic AL, Gealy R, Liskay RM (2005) Novel **PMS1** alleles preferentially affect the repair of primer strand loops during DNA replication. Mol Cell Biol 25: 9221–9231.

27. Marsischky GT, Kolodner RD (1997) Biochemical characterization of the interaction between the **Saccharomyces cerevisiae** MSH2-MSH6 complex and mispaired bases in DNA. J Biol Chem 274: 26668–26672.

28. Rodriguez GP, Romanova NV, Bao G, Rouf NC, Kow YW, et al. (2012) Mismatch repair dependent mutagenesis in nondividing cells. Proc Natl Acad Sci USA 109: 6153–6158.

29. Paspalas CA, Aspelund J, Liskay RM (1997) Functional domains of the **Saccharomyces cerevisiae** Mlh1p and Pms1p DNA mismatch repair proteins and their relevance to human hereditary nonpolyposis colorectal cancer-associated mutations. Mol Cell Biol 17: 4465–4473.

30. Gupta S, Gellert M, Yang W (2012) Mechanism of mismatch recognition revealed by human MutSβ bound to unpaired DNA loops. Nat Struct Mol Biol 19: 72–78.

31. Dowen JM, Putnam CD, Kolodner RD (2010) Functional studies and homology modeling of Msh2-Msh3 predict that mismatch recognition involves DNA bending and strand separation. Mol Cell Biol 30: 3264–3270.
35. Nishant KT, Phys AJ, Alani E (2008) A mutation in the putative MLH3 endonuclease domain confers a defect in both mismatch repair and meiosis in Saccharomyces cerevisiae. Genetics 179: 747–755.

36. Hombauer H, Campbell CS, Smith CE, Desai A, Kolodner RD (2011) Visualization of eukaryotic DNA mismatch repair reveals distinct recognition and repair intermediates. Cell 147: 1040–1053.

37. Moldovan GL, Pfander B, Jentsch S (2007) PCNA, the maestro of the replication fork. Cell 129: 665–679.

38. Lee SD, Alani E (2000) Analysis of interactions between mismatch repair initiation factors and the replication processivity factor PCNA. J Mol Biol 355: 175–184.

39. Johnson RE, Kovalski GK, Guzder SN, Amin NS, Holm C, et al. (1996) Evidence for involvement of yeast proliferating cell nuclear antigen in DNA mismatch repair. J Biol Chem 271: 27907–27910.

40. Umar A, Buermeyer AB, Simon JA, Thomas DC, Clark AB, et al. (1996) Requirement for PCNA in DNA mismatch repair at a step preceding DNA resynthesis. Cell 67: 65–73.

41. Chen C, Merrill BJ, Lau PJ, Holm C, Kolodner RD (1999) Saccharomyces cerevisiae pol30 (proliferating cell nuclear antigen) mutations impair replication fidelity and mismatch repair. Mol Cell Biol 19: 7801–7815.

42. Pluciennik A, Dzantiev L, Iyer RR, Constantin N, Kadervo FA, et al. (2010) PCNA function in the activation and strand direction of MutLX endonuclease in mismatch repair. Proc Natl Acad Sci USA 107: 16066–16071.

43. Amin NS, Nguyen MN, Oh S, Kolodner RD (2001) euk-dependent mutator mutations: Model system for studying functional interactions in mismatch repair. Mol Cell Biol 21: 5142–5155.

44. Bowers J, Tran PT, Joshi A, Lisay RM, Alani E (2001) MSH-MLH complexes formed at a DNA mismatch are disrupted by the PCNA sliding clamp. J Mol Biol 306: 957–968.

45. Clark AB, Valle F, Drotschmann K, Gary RK, Kunkel TA (2000) Functional interaction of proliferating cell nuclear antigen with MSH2-MSH6 and MSH2-MSH3 complexes. J Biol Chem 275: 36498–36501.

46. Flores-Rezas H, Clark D, Kolodner RD (2000) Proliferating cell nuclear antigen and Msh2p-Msh6p interact to form an active mispair recognition complex. Nature Genet 26: 373–378.

47. Hargreaves VV, Putnam CD, Kolodner RD (2012) Engineered diolide-forming amino acid substitutions interfere with a conformational change in the mismatch repair complex Msh2-Msh6 required for mismatch repair. J Biol Chem 287: 14232–14244.

48. Lau PJ, Flores-Rezas H, Kolodner RD (2002) Isolation and characterization of new proliferating cell nuclear antigen (POL30) mutator mutations that are defective in DNA mismatch repair. Mol Cell Biol 22: 6669–6680.

49. Iyer RR, Pluciennik A, Genschel J, Tsiu MS, Besse LS, et al. (2010) MutLX and proliferating cell nuclear antigen share binding sites on MutSβ. J Biol Chem 285: 11730–11739.

50. Lau PJ, Kolodner RD (2003) Transfer of the MSH2-MSH6 complex from proliferating cell nuclear antigen to mispaired bases in DNA. J Biol Chem 278: 14–17.

51. Kumar P, Nagarajaram HA (2012) A study on mutational dynamics of simple sequence repeats in relation to mismatch repair system in prokaryotic genomes. J Mol Evol 74: 127–139.

52. Shell SS, Pumam CD, Kolodner RD (2007) Chimeric Saccharomyces cerevisiae Msh6 protein with an Msh3 mispair-binding domain combines properties of both proteins. Proc Natl Acad Sci USA 104: 10956–10961.

53. Lopez CA, Cleary JD, Pearson CE (2010) Repeat instability as the basis for human diseases and as a potential target for therapy. Nat Rev Mol Cell Biol 11: 165–170.

54. Shah SN, Hile SE, Eckert KA (2010) Defective mismatch repair, microsatellite mutation bias, and variability in clinical cancer phenotypes. Cancer Res 70: 12593–12598.

55. Ezzati-zadeh V, Pinto RM, Sandi C, Sandi M, Al-Mahdawi S, et al. (2012) The mismatch repair system protects against intergenerational GAA repeat instability in a Friedrich ataxia mouse model. Neurobiol Dis 46: 165–171.

56. Lang WH, Coats JE, Majak J, Hura GL, Liu Y, et al. (2011) Conformational trapping of mismatch recognition complex MSH2/MSH3 on repair-resistant DNA loops. Proc Natl Acad Sci U S A 108: E837–E844.

57. Paingrahi GB, Sear MM, Simard JP, Gudgeon OR, Pearson CE (2010) Isolated short CTG/CAG DNA dip-outs are repaired efficiently by bMutSβ, but clustered dip-outs are poorly repaired. Proc Natl Acad Sci USA 107: 12593–12598.

58. Guo J, Zheng L, Liu W, Wang X, Wang Z, et al. (2011) Frequent truncating mutation of FEAM induces mitochondrial DNA depletion and apoptotic resistance in microsatellite-unstable colorectal cancer. Cancer Res 71: 2978–2987.

59. Duval A, Hamelin R (2002) Mutations at coding repeat sequences in mismatch repair-deficient human cancers: Toward a new concept of target genes for instability. Cancer Res 62: 2447–2454.

60. Hegan DC, Narayanan L, Jirik FR, Edelmann W, Lisay RM, et al. (2006) Differing patterns of genetic instability in mice deficient in the mismatch repair genes Pms2, Mlh1, Msh2, Msh3, and Msh6. Carcinogen 27: 2402–2408.

61. Hargreaves VV, Thomas S, Sandstrom R, Canfield TK, Thurman RE, et al. (2010) Sequencing newly replicated DNA reveals widespread plasticity in human replication timing. Proc Natl Acad Sci USA 107: 139–144.

62. Rothstein R (1990) Targeting, disruption, replacement, and allele rescue: integrative DNA transformation in yeast. Methods Enzymol 194: 3–21.

63. Datta A, Jinks-Robertson S (1995) Association of increased spontaneous mutation rates with high levels of transcription in yeast. Science 268: 1616–1619.

64. Abulhoul AL, Minesinger BK, Jinks-Robertson S (2008) The effect of sequence context on spontaneous Pol- dependent mutagenesis in Saccharomyces cerevisiae. Nucleic Acids Res 36: 2082–2093.

65. Winzeler EA, Shoemaker DD, Astromoff A, Liang H, Anderson K, et al. (1999) Functional characterization of the S. cerevisiae genome by gene deletion and parallel analysis. Science 285: 901–906.

66. Storici F, Lewis LK, Resnick MA (2001) In vivo site-directed mutagenesis using oligonucleotides. Nat Biotechnol 19: 773–776.

67. Rodriguez GP, Song JB, Crouse GF (2012) Transformation with oligonucleotides creating clustered changes in the yeast genome. PLoS ONE 7: e29265.

68. Sherman F (1994) Getting started with yeast. Methods Enzymol 194: 3–21.

69. Sikorski RS, Hieter P (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in Saccharomyces cerevisiae. Genetics 122: 19–27.