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Non-canonical actions of mismatch repair

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Abstract

At the heart of the mismatch repair (MMR) system are proteins that recognize mismatches in DNA. Such mismatches can be mispairs involving normal or damaged bases or insertion/deletion loops due to strand misalignment. When such mispairs are generated during replication or recombination, MMR will direct removal of an incorrectly paired base or block recombination between nonidentical sequences. However, when mispairs are recognized outside the context of replication, proper strand discrimination between old and new DNA is lost, and MMR can act randomly and mutagenically on mispaired DNA. Such non-canonical actions of MMR are important in somatic hypermutation and class switch recombination, expansion of triplet repeats, and potentially in mutations arising in nondividing cells. MMR involvement in damage recognition and signaling is complex, with the end result likely dependent on the amount of DNA damage in a cell.

Keywords

Mispaired base; Replication; Strand discrimination; DNA damage; Mutagenesis; DNA replication fidelity

1. Introduction

In order to review non-canonical actions of mismatch repair (MMR), it is first necessary to understand its canonical functions. There are many extensive reviews on MMR, in this special issue and elsewhere [1–8], but defining the canonical functions of MMR is not as simple as it might at first seem to be. Like many biological activities, MMR was named for its first discovered function. Any basic description of MMR will begin (and usually end) with an explanation of its role in recognition and subsequent elimination of mismatched base pairs formed during replication and the fact that MMR activity increases the accuracy of replication by several orders of magnitude. There is also usually some short description of how the incorrectly paired base is determined, most frequently using the example of elimination of the unmethylated strand of DNA in *E. coli*. Although not incorrect, that

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description is very incomplete and focuses on what is probably the least important of the functions of MMR in a normal cell: the recognition of a mismatched base pair of undamaged bases.

It was first found in *E. coli* that MMR also recognized and repaired small loops of 1–4 bp [9]. Much subsequent work has revealed the importance of MMR in suppressing insertion/deletion (in/del) loops that are usually the result of slipped mispairing [10, 11]. It is now clear that the mutator phenotypes of loss of MMR on frameshift mutations, and more generally in/del mutations, are much greater than for base pair substitutions. There are two mismatch recognition complexes in many eukaryotes, MutS α and MutS β , and we have recently suggested that the existence of MutS β is likely due in large part to its role in suppressing in/del mutations, particular in/del mispairs that would lead to deletions [12].

Although MMR is certainly able to recognize and repair mispairs of normal bases formed during replication, in cells with normally functioning proofreading, this activity is not likely to be one of its major functions. My lab for example recently demonstrated, in an assay system in yeast specific for base pair mutations, that loss of MMR resulted in a relatively small increase in base pair substitutions [13] but was extremely important in the absence of proofreading [14]. It has been known for many years that MMR could recognize mispairs containing damaged bases [3–6, 15, 16], and we suggested that MMR has a much more important role in suppressing mutations due to damaged bases than for mispairs containing only undamaged bases [13, 17]. In addition to damaged bases, it has also been shown that MMR can target mispairs involving ribonucleotides [18]. We also demonstrated that when endogenous levels of reactive oxygen species were increased by elimination of Sod1, base pair mutation rates generally increased by an order of magnitude or more in the absence of MMR [13]. A recent genome-wide analysis of spontaneous mutations in *E. coli* showed a 100-fold increase in base pair substitutions in MMR-defective compared to wild type strains [19]. However, in such analyses there is generally no way in which to determine how much of that increase was due to mispairing of normal bases, and many of the increased mutations could be explained by misincorporations due to damaged bases [13, 19]. Eukaryotic organisms tend to have more, and longer, sequences of simple repeats than do prokaryotic organisms [12] and the relative effect of loss of MMR on such repeats is typically much larger than for base pair substitutions. For example, loss of MMR can increase instability of homopolymer runs in yeast from 5,000-fold [20] to 10,000-fold [21].

From the studies cited above, it was not clear in what way MMR was acting to suppress mutations due to DNA lesions. As indicated in Fig. 1A, if a mismatch is formed during replication by insertion of some type of mispair, whether due to mispaired bases, in/del loops, or by incorporation of a damaged base, recognition by MMR will lead to excision of the primer strand and consequent removal of the mispair. In its replicative repair functions, MMR cannot repair damage to the template replicating strand, as shown in Fig. 1B. Excision of the primer strand DNA will still leave the lesion in the template strand. For such situations, prevention of mutations by MMR would have to work through monitoring the fidelity of base insertion opposite a damaged base or by eliminating cells with damaged template strands. For a specific type of template damage it was shown that MutS α was responsible for removal of adenine misincorporated opposite a template 8-oxoG [22],

illustrating that at least in some cases, MMR can suppress mutations by recognition of a mispair opposite a lesion. This type of mispair recognition could potentially be effective for lesion bypass by either normal replicative or translesion polymerases.

Thus it appears that in terms of repair and prevention of mutations, the important canonical functions of MMR are for repair of in/del loops and mismatches involving damaged bases. Clearly, mismatches involving normal bases are also repaired, and such MMR activity becomes much more important when proofreading activity is lacking [23–30].

Although effects of MMR on recombination are not part of the repair functions of MMR, it has been clear that MMR is important in recombination, specifically in preventing recombination between sequences that are not completely homologous, as a speciation and rearrangement barrier [1–5, 10, 11, 31, 32]. The central idea is that mismatches in recombination intermediates are recognized by MMR proteins and such intermediates are blocked from completing recombination. This topic is reviewed by Tlām and Lebbink in this special issue [33].

In its function in both repair and (anti)recombination, mismatches are first recognized. The next step is to discriminate between new and old DNA for repair during replication, or the invading strand in recombination [1–8]. As illustrated in Fig. 1A and B, the primer strand of DNA is then excised. It is still not clear how strand discrimination is achieved in repair. Some bacterial species use strand methylation, but many do not (see Putnam in this special issue [34]). Whether or not strand methylation is used, the presence of a nick seems in many cases sufficient. In eukaryotes, a strand discontinuity can give strand discrimination, Kadyrova and Kadyrov in this special issue and others [35–37], and recently it has been shown that ribonucleotides incorporated into DNA and removed by RNase H2 can serve as one source of nicks [38, 39]. Orientation of the MMR complex by interaction with PCNA remains another possibility for strand discrimination [40, 41]. In recombination, it is likely the recognition of an end of the invading DNA that gives discrimination information. Thus one could consider the canonical functions of MMR as those in which mismatches are recognized and then the primer strand of DNA is correctly recognized and removed.

The question then becomes, what happens when MMR recognizes mismatches, but there are no strand discrimination signals available or there are signals that are recognized as strand discrimination signals, but that do not denote newly replicated or invading DNA? Fig. 1C indicates that in the absence of any strand discrimination signal it is not clear what action MMR would take, but if a nick were to be present in non-replicating DNA, MMR could use that as a signal for strand excision. When MMR acts in those cases, its actions can be viewed as non-canonical. In some cases, such as somatic hypermutation, the MMR system has apparently been coopted to perform what has become an important function. In other cases, non-canonical actions of MMR can be deleterious to the organism.

2. Early reports of non-canonical actions of MMR

2.1. Gene conversion gradients in yeast

In yeast meiosis, there are double-strand breaks (DSBs) that initiate recombination between chromatids of homologous chromosomes. Gene conversion results when the invading strand of DNA is repaired from the donor chromosome such that all strands of the chromatids end up with the same allele; MMR is involved in this process, as the absence of MMR results in aberrant segregation events known as postmeiotic segregation (PMS) [42]. It was observed that gene conversion frequencies were high for markers located near DSBs, but that the frequency of gene conversion events decreased with the distance of markers from a DSB. This phenomenon was explained by proposing that MMR would preferentially use the donor chromosome for repair of markers near the DSB, but would lose directionality as marker distance increased from the DSB, presumably due to the loss of a signal for strand discrimination [43–45]. Another study found that a steep gene conversion gradient at the *ARG4* locus was flattened in the presence of a low activity allele of *MLH1*, presumably due to a loss of proper strand discrimination signals [46].

Although the mechanism of strand discrimination is not known for meiotic recombination, the experiments above are consistent with DNA ends at the site of the DSB being used as that signal. As distances increase from the site of the DSB, there is random repair of the heteroduplex (which gives results that are distinct from the PMS events observed in the absence of MMR). The random repair is a marker of MMR action having lost proper strand discrimination. The role of MMR during meiotic recombination is detailed further by Mannhart and Alani in this issue [47].

2.2 Mouse somatic hypermutation

Given the large increase in mutation rates observed in somatic hypermutation at the immunoglobulin locus, one might have expected the hypermutation process to be moderated by MMR, or that MMR would be excluded from the region of hypermutation [48–54]. Thus it was a particular surprise to find that the presence of MMR increased somatic hypermutation; indeed the group that discovered this effect proposed that during somatic hypermutation, MMR had a strand discrimination bias opposite to that for replicative MMR [55]. A report in that same time frame found that A-T mutations were particularly sensitive to MMR [56]. Mutations at C-G sequences could be explained by the actions of activation-induced cytidine deaminase [52, 54], but it was more difficult to understand the involvement of MMR in somatic hypermutation at A-T sequences.

Recent results have begun to reveal the non-canonical nature of MMR action in somatic hypermutation and class switch recombination [49, 57–59]. This topic is reviewed by Zanotti and Gearhart in this special issue [60]. I will only note here that a central understanding of the role of MMR in this process was the realization that MMR was recruited by DNA containing a lesion, rather than a mismatch occurring during replication. The resulting MMR complex was in a context outside of replication and thus lacked strand directionality and also recruited an error-prone DNA polymerase [58]. Any resulting MMR activity without proper strand discrimination would thus be mutagenic.

2.3. Postulated role of random acting MMR in cancer

In 1995, after the basics of MMR activity had been elucidated in both bacterial and eukaryotic systems, MacPhee speculated that MMR could prove to be mutagenic if it recognized mismatches in the absence of an appropriate strand selection signal [61], which he termed randomly template MMR. He further postulated that such activity could account for the high mutation rates observed in cancer and could explain how mutations could arise in nondividing cells. One aspect of the proposal was that nondividing cells in which mutations arose would have to start replication again, perhaps in response to the mutations. Although there were attractive aspects of this proposal, the only evidence that MacPhee could cite of random acting MMR in eukaryotes was gene conversion in yeast. In addition, the only known involvement of MMR in cancer at that time was the absence of MMR leading to Lynch syndrome [62–65]. New findings relevant to this topic are discussed further below.

2.4 MMR and trinucleotide repeat instability

Over 40 neurological disorders have been linked to expansions of simple repeated sequences, generally trinucleotide repeats [66]. Given the important, canonical, role of MMR in preventing mutations due to slipped mispairing of simple repeats, one would expect that the presence of MMR would reduce repeat expansions leading to disease. However, in the case of trinucleotide repeats (CAG/CTG, CGG/CCG, and GAA/TTC) the opposite is true; MMR proteins, including MutS β , MutL α and possibly MutL γ , have been shown to enhance the expansions of the repeats [66]. The involvement of MMR with trinucleotide repeat instability is reviewed in this special issue by Schmidt and Pearson [67] and will not be further discussed here. It is worth noting that the components of MMR involved with repeat expansion are those involved in loop recognition and likely involve loop recognition outside the context of replication.

3. Recent results demonstrating non-canonical effects of MMR

As indicated in the previous section, there have been various situations in which it appears that MMR was acting in non-canonical ways. However, in the last few years, there have been a variety of experiments, both *in vivo* and *in vitro*, that shed new light on non-canonical activities of MMR [58, 68–72].

3.1 MMR-dependent mutagenesis in nondividing yeast cells

As mentioned above, MacPhee postulated that MMR might be able to act in a random manner on mismatches in nondividing cells [61]. However, the conditions for demonstrating such activity are quite demanding: a mutation that is created in a cell that remains nondividing would never be observed, so the new mutation should be a dominant gain of function mutation that would cause renewed cell growth. In addition, one would want to know that the resulting mutation was created by a mismatch that was randomly resolved by MMR, and not, for example, by some type of inaccurate repair synthesis. My lab developed an assay system for point mutations using mutant alleles of an essential codon in the yeast *TRP5* gene [73]. The assay is exceedingly specific; for any of our *trp5* alleles, Trp⁺ strains

can arise only due to reversion events to the original wild-type sequence [73]. In addition *trp5* strains plated on Trp-medium will not undergo even a second round of replication [71].

Using this assay system, we created mismatches at the mutant allele site by transforming in short oligonucleotides that would place a wild-type sequence in the chromosome on the transcribed or on the non-transcribed strand [71]. As illustrated in Fig. 2, in the absence of MMR, a wild-type sequence placed on the transcribed strand gave many revertants, whereas a wild-type sequence placed on the nontranscribed strand gave very few revertants. In contrast, in the presence of MMR, many revertants were obtained with oligonucleotides creating wild-type sequences on the nontranscribed strand, and relatively fewer revertants with oligonucleotides creating mismatches on the transcribed strand. In the presence of MMR, the mismatch created by the wild-type sequence was randomly corrected: when on the nontranscribed strand yielding many mutations that would allow growth, and when on the transcribed strand reducing the number of chromosomes containing the wild-type sequence [71].

Another experiment more directly mimicked what might occur in nondividing cells. As shown in Fig. 3 we electroporated 8-oxoGTP into the cells containing the same *trp5* allele as in Fig. 2, and plated on Trp-medium. In the absence of MMR, or when dGTP was used instead of 8-oxoG, essentially no revertants were obtained [71]. However, in the presence of MMR, a few revertants were observed after 3 days of incubation on the plate, and revertants continued accumulating over a period of almost two weeks [71]. In this case, 8-oxoG that had been incorporated opposite A remained on the nontranscribed strand after replication; only in the presence of MMR was the 8-oxoG-A mispair randomly corrected by MMR to a wild type sequence that would allow growth of the cell. Moreover, the MMR activity in the nondividing cells could occur after many days of being plated.

Because of the particular nature of the assay used, both of these experiments were able to demonstrate mutations that arose only in the presence of MMR and in a direction counter to a canonical action of MMR (i.e. retention of the mispaired base on the primer strand of replication).

3.2 Non-canonical MMR in other processes

With high levels of DNA damage, it has been shown in numerous experiments that MMR plays an important role in damage signaling and apoptosis; see Li et al. in this special issue and other references [1–5, 16, 69, 72, 74–76].

It is clear that MMR recognizes a variety of lesions when they are present in duplex DNA [3–6, 15]. One pathway of lesion formation in DNA would be through incorporation of damaged nucleotides. There has been extensive study of incorporation of oxidized nucleotides, as well as some study of the incorporation of other types of damaged bases [77–83]. MMR could potentially block such incorporation during replication and has been shown to block incorporation of 8-oxoGMP into DNA [84]. The presence of an entire repair system devoted to the repair of oxidized guanine products in *E. coli*, including an enzyme for cleansing 8-oxoGTP from the nucleotide pool (MutT) [85, 86], and the existence of a similar system in mammals [87] is evidence that the threat of damaged nucleotides can be

significant. It is interesting in this context that yeast appears to lack homologues of MutT and relies on MMR [17, 22].

It is likely, however, that most DNA lesions encountered during replication would be those on the template strand that had not been repaired before the start of replication as illustrated in Fig. 1B. In that case, MMR recognition of these lesions at best could discriminate between formation of correct versus incorrect base pairs if that could be determined, but could not lead to the repair of the lesion. With high levels of DNA damage, it has been shown in numerous experiments that MMR plays an important role in damage signaling and apoptosis although it remains unclear whether the mechanism is through a futile repair model or direct signaling of the lesions through the ATR-Chk1 pathway [1–5, 69, 72, 74–76]. The particular importance of MMR in damage signaling is illustrated by the very large increase in cell survival to high doses of MNNG in cells lacking functional MMR [1–5, 72, 75, 76]. The current status of damage signaling and MMR is reviewed in this issue by Li et al. [16].

It is important to emphasize one point about most of the experiments that have examined the effects of lesion-containing DNA on the cell: those experiments have generally been performed by treating cells with relatively high doses of a mutagen or inactivating some repair system in the cell. Because many repair proteins and translesion DNA polymerases tend to be present in the cell at low levels, some of the observed effects may be due to the indirect effects of exhaustion of cellular components. As one example, in one set of our *trp5* strains, we found that elimination of Ogg1, a glycosylase removing 8-oxoG opposite a C, increased reversion rates by more than an order of magnitude and elimination of MMR in such strains increased reversion rates by an additional order of magnitude (Fig. 4A) [13]. Because all reversion events occurred through the same base pair change at the same locus, the intermediate reversion rates observed in *ogg1* strains suggested a partial deficit of MMR activity. When we used oligonucleotides to introduce one 8-oxoG into the yeast genome, that 8-oxoG was correctly replicated in 98% of wild-type cells examined and over 90% of the time in either the absence of MMR or the absence of the TLS polymerase Pol η (Fig. 4B) [88]. It is important to note that translesion synthesis of the 8-oxoG could occur in the absence of MMR and recruitment of Pol η was presumably invoked by stalling of the normal replicative DNA polymerase [88]; we don't know whether the same would be true in mammalian cells. In the absence of Pol η , the presence of MMR yielded accurate replication [88]. Given the inaccuracy of the replicative polymerases across 8-oxoG, accurate replication would likely have involved several rounds of MMR-directed resynthesis across the 8-oxoG. The difference in results of the experiments in Fig. 4A compared to Fig. 4B is presumably due to the much higher burden of 8-oxoG lesions in the *ogg1* strains. We have also obtained preliminary evidence that MMR can distinguish a C from a T placed opposite an O⁶-methyl-dG and can lead to greatly increased accuracy of bypass, again likely through multiple rounds of translesion synthesis. A few such lesions could thus perhaps be accommodated in a cell, but many O⁶-methyl-dG lesions would probably cause so much replication resynthesis as to give the observed cell death. Such MMR-directed futile cycling has been recently observed in *Xenopus laevis* egg extracts [89].

Lesion-containing DNA can clearly exist outside of replication, and the problem of lesion recognition in that case would be the lack of any proper strand discrimination signal. In addition to demonstrating non-canonical MMR in lymphoid cells, Peña-Díaz et al. found similar activity in other cells [58]. Their conclusion was that recognition of lesions by MMR and recruitment of Pol η by PCNA ubiquitination outside the context of replication in a variety of cell types could lead to MMR actions without proper strand discrimination signals and thus be mutagenic. This action is similar to what we observed with 8-oxoG-containing DNA in nondividing cells [88] and illustrated in Figs. 2 and 3.

In our experiment illustrated in Fig. 2, some base-base mispairs created by the oligonucleotides persisted into G2 phase, even in the presence of MMR, and were repaired without proper strand discrimination [71]. It has recently been found, by analyzing a large dataset from cancer genome sequencing, that mutation rates were reduced in early replicating euchromatin and elevated in late replicating heterochromatic regions [90]. Particularly interesting was the observation that the distribution of mutations with respect to replication timing was much flatter in the absence of MMR than in the presence of MMR [90]. Because MMR is most active in S phase, these results would be consistent with replication-associated mismatches arising early in replication having the most time to be repaired, whereas those arising late having the least time to be repaired. In addition, those mismatches escaping replication-associated MMR might be subject to mutagenic MMR outside the context of replication, further increasing the mutation rate in those regions [90].

3.3 Possible mechanisms of non-canonical MMR

It has been known since the first biochemical studies of eukaryotic MMR that covalently closed circular DNA substrates containing a mismatch were essentially refractory to MMR, whereas a single nick could serve as a strand discrimination signal [35, 36]. Therefore it could be in some circumstances that MMR recognition of a mispair outside the context of replication could remain inactive until the adventitious occurrence of a nick in the vicinity that would then activate repair as indicated in Fig. 1C. Such a process could explain the very long lag times we observed in reversion of nondividing cells that had incorporated 8-oxoGTP into the genome [71].

However, a very important finding was made by Pluciennik et al. in 2010 [91] with additional experiments in 2013 [92]. These papers demonstrated that a covalently closed circular DNA containing a lesion recognized by either MutS α or MutS β and a helix perturbation could support PCNA loading and subsequent MutL α activation. However, the activation of MutL α with subsequent strand cleavage was without strand bias. This work provides a framework for understanding how MMR recognition outside of replication could give rise to MutL α activation that would result in strand removal and subsequent resynthesis that would be random with respect to strand nicking and removal; see the review by Kadyrova and Kadyrov in this special issue [37]. The rate of MutL α activation on covalently closed substrates is much slower than with nicked DNA [92]; it is not clear whether that would be the rate limiting step in our experiments [71].

One of the particular interests in the work of Pluciennik et al. was the possible activation of MMR by extrahelical triplet repeats and thus an explanation for the role of MMR in repeat

expansion [92]. A potentially complicating factor in understanding the role of MMR in repeat expansion is that, at least in yeast, MutS α and MutS β have opposite biases in repair of deletion versus insertion loops [12], perhaps as a consequence of their different interactions with PCNA [93].

4. Summary

Canonical MMR involves recognition of some type of mispair in the context of an appropriate strand discrimination signal—for either the replicating strand or the invading strand in recombination. However, when the relevant signal is not present, such as mismatches formed far away from the invading end of a recombination structure or when mismatches are recognized outside the context of a replication fork, activation of MMR can result in mutagenic activity; such activity has been observed in both yeast and mammalian cells. In some cases, this non-canonical activity has been “hijacked” by the cell for discrete purposes, with somatic hypermutation and class switch recombination the most obvious example. Recognition of a mispair with a template-strand lesion in the context of replication cannot lead to repair of the lesion by MMR. However, in many cases MMR could monitor the incorporation of the correct base. For situations in which there is extensive DNA damage in a cell, it is likely that both MMR and translesion DNA synthesis and repair functions could be overwhelmed; in those cases MMR recognition of the damage could lead to a cell cycle arrest or apoptosis.

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References

1. Jiricny J. Postreplicative mismatch repair. *Cold Spring Harb Perspect Biol.* 2013; 5:a012633. [PubMed: 23545421]
2. Li GM. Mechanisms and functions of DNA mismatch repair. *Cell Res.* 2008; 18:85–98. [PubMed: 18157157]
3. Modrich P. Mechanisms in eukaryotic mismatch repair. *J Biol Chem.* 2006; 281:30305–30309. [PubMed: 16905530]
4. Jiricny J. The multifaceted mismatch-repair system. *Nat Rev Mol Cell Biol.* 2006; 7:335–346. [PubMed: 16612326]
5. Iyer RR, Pluciennik A, Burdett V, Modrich PL. DNA mismatch repair: functions and mechanisms. *Chem Rev.* 2006; 106:302–323. [PubMed: 16464007]
6. Kunkel TA, Erie DA. DNA mismatch repair. *Annu Rev Biochem.* 2005; 74:681–710. [PubMed: 15952900]
7. Kolodner RD. Introduction to MMR from a historical and personal perspective. *DNA Repair.* 2016 this issue.
8. Goellner EM, Putnam CD, Kolodner RD. Exonuclease 1-dependent and independent mismatch repair. *DNA Repair (Amst).* 2015; 32:24–32. [PubMed: 25956862]
9. Parker BO, Marinus MG. Repair of DNA heteroduplexes containing small heterologous sequences in *Escherichia coli*. *Proc Natl Acad Sci USA.* 1992; 89:1730–1734. [PubMed: 1542666]
10. Harfe BD, Jinks-Robertson S. DNA mismatch repair and genetic instability. *Annu Rev Genet.* 2000; 34:359–399. [PubMed: 11092832]

11. Harfe BD, Jinks-Robertson S. Mismatch repair proteins and mitotic genome stability. *Mutat Res.* 2000; 451:151–167. [PubMed: 10915870]
12. Romanova NV, Crouse GF. Different roles of eukaryotic MutS and MutL complexes in repair of small insertion and deletion loops in yeast. *PLoS Genet.* 2013; 9:e1003920. [PubMed: 24204320]
13. Shockley AH, Doo DW, Rodriguez GP, Crouse GF. Oxidative damage and mutagenesis in *Saccharomyces cerevisiae*: genetic studies of pathways affecting replication fidelity of 8-oxoguanine. *Genetics.* 2013; 195:359–367. [PubMed: 23893481]
14. Flood CL, Rodriguez GP, Bao G, Shockley AH, Kow YW, Crouse GF. Replicative DNA Polymerase δ but Not ϵ Proofreads Errors in *Cis* and in *Trans*. *PLoS Genet.* 2015; 11:e1005049. [PubMed: 25742645]
15. Stojic L, Brun R, Jiricny J. Mismatch repair and DNA damage signalling. *Mutation Research: DNA Repair.* 2004; 3:1091–1101.
16. Li Z, Pearlman AH, Hsieh P. MMR and the DNA Damage Response. *DNA Repair.* 2016 this issue.
17. Earley MC, Crouse GF. The role of mismatch repair in the prevention of base pair mutations in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA.* 1998; 95:15487–15491. [PubMed: 9860995]
18. Shen Y, Koh KD, Weiss B, Storici F. Mismatched rNMPs in DNA are mutagenic and are targets of mismatch repair and RNases H. *Nat Struct Mol Biol.* 2012; 19:98–104. [PubMed: 22139012]
19. Lee H, Popodi E, Tang H, Foster PL. Rate and molecular spectrum of spontaneous mutations in the bacterium *Escherichia coli* as determined by whole-genome sequencing. *Proc Natl Acad Sci U S A.* 2012; 109:E2774–E2783. [PubMed: 22991466]
20. Sia EA, Kokoska RJ, Dominska M, Greenwell P, Petes TD. Microsatellite instability in yeast: Dependence on repeat unit size and DNA mismatch repair genes. *Mol Cell Biol.* 1997; 17:2851–2858. [PubMed: 9111357]
21. Tran HT, Keen JD, Krickler M, Resnick MA, Gordenin DA. Hypermutability of homonucleotide runs in mismatch repair and DNA polymerase proofreading yeast mutants. *Mol Cell Biol.* 1997; 17:2859–2865. [PubMed: 9111358]
22. Ni TT, Marsischky GT, Kolodner RD. MSH2 and MSH6 are required for removal of adenine misincorporated opposite 8-oxo-guanine in *S. cerevisiae*. *Mol Cell.* 1999; 4:439–444. [PubMed: 10518225]
23. Morrison A, Johnson AL, Johnston LH, Sugino A. Pathway correcting DNA replication errors in *Saccharomyces cerevisiae*. *EMBO J.* 1993; 12:1467–1473. [PubMed: 8385605]
24. Minnick DT, Pavlov YI, Kunkel TA. The fidelity of the human leading and lagging strand DNA replication apparatus with 8-oxodeoxyguanosine triphosphate. *Nucleic Acids Res.* 1994; 22:5658–5664. [PubMed: 7838719]
25. Morrison A, Sugino A. The 3'→5' exonucleases of both DNA polymerases δ and ϵ participate in correcting errors of DNA replication in *Saccharomyces cerevisiae*. *Mol Gen Genet.* 1994; 242:289–296. [PubMed: 8107676]
26. Tran HT, Gordenin DA, Resnick MA. The 3'→5' exonucleases of DNA polymerases δ and ϵ and the 5'→3' exonuclease Exo1 have major roles in postreplication mutation avoidance in *Saccharomyces cerevisiae*. *Mol Cell Biol.* 1999; 19:2000–2007. [PubMed: 10022887]
27. Greene CN, Jinks-Robertson S. Spontaneous frameshift mutations in *Saccharomyces cerevisiae*: Accumulation during DNA replication and removal by proofreading and mismatch repair activities. *Genetics.* 2001; 159:65–75. [PubMed: 11560887]
28. Jin YH, Garg P, Stith CMW, Al Refai H, Sterling JF, Murray LJW, Kunkel TA, Resnick MA, Burgers PM, Gordenin DA. The multiple biological roles of the 3'→5' exonuclease of *Saccharomyces cerevisiae* DNA polymerase δ require switching between the polymerase and exonuclease domains. *Mol Cell Biol.* 2005; 25:461–471. [PubMed: 15601866]
29. Albertson TM, Ogawa M, Bugni JM, Hays LE, Chen Y, Wang Y, Treuting PM, Heddle JA, Goldsby RE, Preston BD. DNA polymerase ϵ and δ proofreading suppress discrete mutator and cancer phenotypes in mice. *Proc Natl Acad Sci USA.* 2009; 106:17101–17104. [PubMed: 19805137]
30. Pavlov YI, Shcherbakova PV. DNA polymerases at the eukaryotic fork-20 years later. *Mutat Res.* 2010; 685:45–53. [PubMed: 19682465]

31. George CM, Alani E. Multiple cellular mechanisms prevent chromosomal rearrangements involving repetitive DNA. *Crit Rev Biochem Mol Biol.* 2012; 47:297–313. [PubMed: 22494239]
32. Evans E, Alani E. Roles for mismatch repair factors in regulating genetic recombination. *Mol Cell Biol.* 2000; 20:7839–7844. [PubMed: 11027255]
33. Tham KC, Lebbink J. Functions of MMR in regulating genetic recombination. *DNA Repair.* 2016 this issue.
34. Putnam CD. Evolution of the methyl directed MMR system in *Escherichia coli*. *DNA Repair.* 2016 this issue.
35. Holmes J Jr, Clark S, Modrich P. Strand-specific mismatch correction in nuclear extracts of human and *Drosophila melanogaster* cell lines. *Proc Natl Acad Sci USA.* 1990; 87:5837–5841. [PubMed: 2116007]
36. Thomas DC, Roberts JD, Kunkel TA. Heteroduplex repair in extracts of human HeLa cells. *J Biol Chem.* 1991; 266:3744–3751. [PubMed: 1995629]
37. Kadyrova LY, Kadyrov FA. Endonuclease activities of MutL α and its homologs in MMR. *DNA Repair.* 2016 this issue.
38. Ghodgaonkar MM, Lazzaro F, Olivera-Pimentel M, Artola-Boran M, Cejka P, Reijns MA, Jackson AP, Plevani P, Muzi-Falconi M, Jiricny J. Ribonucleotides misincorporated into DNA act as strand-discrimination signals in eukaryotic mismatch repair. *Mol Cell.* 2013; 50:323–332. [PubMed: 23603115]
39. Lujan SA, Williams JS, Clausen AR, Clark AB, Kunkel TA. Ribonucleotides are signals for mismatch repair of leading-strand replication errors. *Mol Cell.* 2013; 50:437–443. [PubMed: 23603118]
40. Umar A, Buermeier AB, Simon JA, Thomas DC, Clark AB, Liskay RM, Kunkel TA. Requirement for PCNA in DNA mismatch repair at a step preceding DNA resynthesis. *Cell.* 1996; 87:65–73. [PubMed: 8858149]
41. Johnson RE, Kovvali GK, Guzder SN, Amin NS, Holm C, Habraken Y, Sung P, Prakash L, Prakash S. Evidence for involvement of yeast proliferating cell nuclear antigen in DNA mismatch repair. *J Biol Chem.* 1996; 271:27987–27990. [PubMed: 8910404]
42. Williamson MS, Game JC, Fogel S. Meiotic gene conversion mutants in *Saccharomyces cerevisiae*. I Isolation and characterization of *pms1-1* and *pms1-2*. *Genetics.* 1985; 110:609–646. [PubMed: 3896926]
43. Reenan RAG, Kolodner RD. Characterization of insertion mutations in the *Saccharomyces cerevisiae* *MSH1* and *MSH2* genes: evidence for separate mitochondrial and nuclear functions. *Genetics.* 1992; 132:975–985. [PubMed: 1334021]
44. Alani E, Reenan RAG, Kolodner RD. Interaction between mismatch repair and genetic recombination in *Saccharomyces cerevisiae*. *Genetics.* 1994; 137:19–39. [PubMed: 8056309]
45. Detloff P, White MA, Petes TD. Analysis of a gene conversion gradient at the *HIS4* locus in *Saccharomyces cerevisiae*. *Genetics.* 1992; 132:113–123. [PubMed: 1398048]
46. Argueso JL, Kijas AW, Sarin S, Heck J, Waase M, Alani E. Systematic mutagenesis of the *Saccharomyces cerevisiae* *MLH1* gene reveals distinct roles for Mlh1p in meiotic crossing over and in vegetative and meiotic mismatch repair. *Mol Cell Biol.* 2003; 23:873–886. [PubMed: 12529393]
47. Manhart CM, Alani E. Roles for MMR family proteins in promoting meiotic crossing over. *DNA Repair.* 2016 this issue.
48. Saribasak H, Gearhart PJ. Does DNA repair occur during somatic hypermutation? *Semin Immunol.* 2012; 24:287–292. [PubMed: 22728014]
49. Chahwan R, Edelmann W, Scharff MD, Roa S. Mismatch-mediated error prone repair at the immunoglobulin genes. *Biomedicine & Pharmacotherapy.* 2011; 65:529–536. [PubMed: 22100214]
50. Chang DJ, Cimprich KA. DNA damage tolerance: when it's OK to make mistakes. *Nat Chem Biol.* 2009; 5:82–90. [PubMed: 19148176]
51. Slean MM, Panigrahi GB, Ranum LP, Pearson CE. Mutagenic roles of DNA “repair” proteins in antibody diversity and disease-associated trinucleotide repeat instability. *DNA Repair (Amst).* 2008; 7:1135–1154. [PubMed: 18485833]

52. Peled JU, Kuang FL, Iglesias-Ussel MD, Roa S, Kalis SL, Goodman MF, Scharff MD. The biochemistry of somatic hypermutation. *Annu Rev Immunol.* 2008; 26:481–511. [PubMed: 18304001]
53. Teng G, Papavasiliou FN. Immunoglobulin somatic hypermutation. *Annu Rev Genet.* 2007; 41:107–120. [PubMed: 17576170]
54. Di Noia JM, Neuberger MS. Molecular mechanisms of antibody somatic hypermutation. *Annu Rev Biochem.* 2007; 76:1–22. [PubMed: 17328676]
55. Cascalho M, Wong J, Steinberg C, Wabl M. Mismatch repair co-opted by hypermutation. *Science.* 1998; 279:1207–1210. [PubMed: 9469811]
56. Rada C, Ehrenstein MR, Neuberger MS, Milstein C. Hot spot focusing of somatic hypermutation in MSH2-deficient mice suggests two stages of mutational targeting. *Immunity.* 1998; 9:135–141. [PubMed: 9697843]
57. Zivojnovic M, Delbos F, Girelli Zubani G, Jule A, Alcais A, Weill JC, Reynaud CA, Storck S. Somatic hypermutation at A/T-rich oligonucleotide substrates shows different strand polarities in Ung-deficient or -proficient backgrounds. *Mol Cell Biol.* 2014; 34:2176–2187. [PubMed: 24710273]
58. Pena-Diaz J, Bregenhorn S, Ghodgaonkar M, Follonier C, Artola-Boran M, Castor D, Lopes M, Sartori AA, Jiricny J. Noncanonical mismatch repair as a source of genomic instability in human cells. *Mol Cell.* 2012; 47:669–680. [PubMed: 22864113]
59. Chahwan R, Edelmann W, Scharff MD, Roa S. AIDing antibody diversity by error-prone mismatch repair. *Semin Immunol.* 2012; 24:293–300. [PubMed: 22703640]
60. Zanotti KJ, Gearhart PJ. Antibody diversification caused by abortive MMR and promiscuous DNA polymerases. *DNA Repair.* 2016 this issue.
61. MacPhee DG. Mismatch repair, somatic mutations, and the origins of cancer. *Cancer Res.* 1995; 55:5489–5492. [PubMed: 7585619]
62. Fishel R, Lescoe MK, Rao MRS, Copeland NG, Jenkins NA, Garber J, Kane M, Kolodner R. The human mutator gene homolog *MSH2* and its association with hereditary nonpolyposis colon cancer. *Cell.* 1993; 75:1027–1038. [PubMed: 8252616]
63. Leach FS, Nicolaides NC, Papadopoulos N, Liu B, Jen J, Parsons R, Peltomäki P, Sistonen P, Aaltonen LA, Nyström-Lahti M, Guan X-Y, Zhang J, Meltzer PS, Yu J-W, Kao F-T, Chen DJ, Cerosaletti KM, Fournier REK, Todd S, Lewis T, Leach RJ, Naylor SL, Weissenbach J, Mecklin J-P, Järvinen H, Petersen GM, Hamilton SR, Green J, Jass J, Watson P, Lynch HT, Trent JM, De la Chapelle A, Kinzler KW, Vogelstein B. Mutations of a *mutS* homolog in hereditary nonpolyposis colorectal cancer. *Cell.* 1993; 75:1215–1225. [PubMed: 8261515]
64. Bronner CE, Baker SM, Morrison PT, Warren G, Smith LG, Lescoe MK, Kane M, Earabino C, Lipford J, Lindblom A, Tannergård P, Bollag RJ, Godwin AR, Ward DC, Nordenskjöld M, Fishel R, Kolodner R, Liskay RM. Mutation in the DNA mismatch repair gene homologue *hMLH1* is associated with hereditary non-polyposis colon cancer. *Nature.* 1994; 368:258–261. [PubMed: 8145827]
65. Papadopoulos N, Nicolaides NC, Wei Y-F, Ruben SM, Carter KC, Rosen CA, Haseltine WA, Fleischmann RD, Fraser CM, Adams MD, Venter JC, Hamilton SR, Petersen GM, Watson P, Lynch HT, Peltomäki P, Mecklin J-P, De la Chapelle A, Kinzler KW, Vogelstein B. Mutation of a *mutL* homolog in hereditary colon cancer. *Science.* 1994; 263:1625–1629. [PubMed: 8128251]
66. Pearson CE, Nichol Edamura K, Cleary JD. Repeat instability: mechanisms of dynamic mutations. *Nat Rev Genet.* 2005; 6:729–742. [PubMed: 16205713]
67. Schmidt M, Pearson CE. MMR and trinucleotide repeat instability. *DNA Repair.* 2016 this issue.
68. Bak ST, Sakellariou D, Pena-Diaz J. The dual nature of mismatch repair as antimutator and mutator: for better or for worse. *Front Genet.* 2014; 5:287. [PubMed: 25191341]
69. Pena-Diaz J, Jiricny J. Mammalian mismatch repair: error-free or error-prone? *Trends Biochem Sci.* 2012; 37:206–214. [PubMed: 22475811]
70. Crouse GF. Mismatch repair outside of replication. *Cell Cycle.* 2012; 11:2773–2774. [PubMed: 22801536]

71. Rodriguez GP, Romanova NV, Bao G, Rouf NC, Kow YW, Crouse GF. Mismatch repair dependent mutagenesis in nondividing cells. *Proc Natl Acad Sci USA*. 2012; 109:6153–6158. [PubMed: 22474380]
72. Edelbrock MA, Kaliyaperumal S, Williams KJ. Structural, molecular and cellular functions of MSH2 and MSH6 during DNA mismatch repair, damage signaling and other noncanonical activities. *Mutat Res*. 2013; 743–744:53–66.
73. Williams T-M, Fabbri RM, Reeves JW, Crouse GF. A new reversion assay for measuring all possible base pair substitutions in *Saccharomyces cerevisiae*. *Genetics*. 2005; 170:1423–1426. [PubMed: 15911571]
74. Martin LM, Marples B, Coffey M, Lawler M, Lynch TH, Hollywood D, Marignol L. DNA mismatch repair and the DNA damage response to ionizing radiation: Making sense of apparently conflicting data. *Cancer Treat Rev*. 2010; 36:518–527. [PubMed: 20413225]
75. Hsieh P, Yamane K. DNA mismatch repair: Molecular mechanism, cancer, and ageing. *Mech Ageing Dev*. 2008; 129:391–407. [PubMed: 18406444]
76. O'Brien V, Brown R. Signalling cell cycle arrest and cell death through the MMR System. *Carcinogenesis*. 2006; 27:682–692. [PubMed: 16332722]
77. Ventura I, Russo MT, De LG, Bignami M. Oxidized purine nucleotides, genome instability and neurodegeneration. *Mutat Res*. 2010; 703:59–65. [PubMed: 20601098]
78. Sakumi K, Abolhassani N, Behmanesh M, Iyama T, Tsuchimoto D, Nakabeppu Y. ITPA protein, an enzyme that eliminates deaminated purine nucleoside triphosphates in cells. *Mutat Res*. 2010; 703:43–50. [PubMed: 20601097]
79. Rai P. Oxidation in the nucleotide pool, the DNA damage response and cellular senescence: Defective bricks build a defective house. *Mutat Res*. 2010; 703:71–81. [PubMed: 20673809]
80. Nakabeppu Y, Oka S, Sheng Z, Tsuchimoto D, Sakumi K. Programmed cell death triggered by nucleotide pool damage and its prevention by MutT homolog-1 (MTH1) with oxidized purine nucleoside triphosphatase. *Mutat Res*. 2010; 703:51–58. [PubMed: 20542142]
81. Katafuchi A, Nohmi T. DNA polymerases involved in the incorporation of oxidized nucleotides into DNA: their efficiency and template base preference. *Mutat Res*. 2010; 703:24–31. [PubMed: 20542140]
82. Kamiya H. Mutagenicity of oxidized DNA precursors in living cells: Roles of nucleotide pool sanitization and DNA repair enzymes, and translesion synthesis DNA polymerases. *Mutat Res*. 2010; 703:32–36. [PubMed: 20542139]
83. Henderson PT, Evans MD, Cooke MS. Salvage of oxidized guanine derivatives in the (2'-deoxy)ribonucleotide pool as source of mutations in DNA. *Mutat Res*. 2010; 703:11–17. [PubMed: 20833264]
84. Colussi C, Parlanti E, Degan P, Aquilina G, Barnes D, Macpherson P, Karran P, Crescenzi M, Dogliotti E, Bignami M. The mammalian mismatch repair pathway removes DNA 8-oxodGMP incorporated from the oxidized dNTP pool. *Curr Biol*. 2002; 12:912–918. [PubMed: 12062055]
85. Michaels ML, Tchou J, Grollman AP, Miller JH. A repair system for 8-Oxo-7,8-dihydrodeoxyguanine. *Biochemistry*. 1992; 31:10964–10968. [PubMed: 1445834]
86. Maki H, Sekiguchi M. MutT protein specifically hydrolyses a potent mutagenic substrate for DNA synthesis. *Nature*. 1992; 355:273–275. [PubMed: 1309939]
87. Dizdaroglu M. Oxidatively induced DNA damage: mechanisms, repair and disease. *Cancer Lett*. 2012; 327:26–47. [PubMed: 22293091]
88. Rodriguez GP, Song JB, Crouse GF. *In Vivo* bypass of 8-oxodG. *PLoS Genet*. 2013; 9:e1003682. [PubMed: 23935538]
89. Olivera Harris M, Kallenberger L, Artola Boran M, Enoiu M, Costanzo V, Jiricny J. Mismatch repair-dependent metabolism of O⁶-methylguanine-containing DNA in *Xenopus laevis* egg extracts. *DNA Repair (Amst)*. 2015; 28:1–7. [PubMed: 25697728]
90. Supek F, Lehner B. Differential DNA mismatch repair underlies mutation rate variation across the human genome. *Nature*. 2015; 521:81–84. [PubMed: 25707793]
91. Pluciennik A, Dzantiev L, Iyer RR, Constantin N, Kadyrov FA, Modrich P. PCNA function in the activation and strand direction of MutLa endonuclease in mismatch repair. *Proc Natl Acad Sci USA*. 2010; 107:16066–16071. [PubMed: 20713735]

92. Pluciennik A, Burdett V, Baitinger C, Iyer RR, Shi K, Modrich P. Extrahelical (CAG)/(CTG) triplet repeat elements support proliferating cell nuclear antigen loading and MutLa endonuclease activation. *Proc Natl Acad Sci U S A*. 2013; 110:12277–12282. [PubMed: 23840062]
93. Lau PJ, Flores-Rozas H, Kolodner RD. Isolation and characterization of new proliferating cell nuclear antigen (*POL30*) mutator mutants that are defective in DNA mismatch repair. *Mol Cell Biol*. 2002; 22:6669–6680. [PubMed: 12215524]

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Highlights

- Non-canonical actions of MMR occur outside the context of normal replication
- Non-canonical MMR can occur in nondividing cells and can be mutagenic
- Non-canonical MMR appears to be important in somatic hypermutation
- Non-canonical MMR could be a source of mutation in late replicating DNA
- Non-canonical MMR could be a source of mutations that lead to some tumors

Fig. 1.

Variable effects of MMR recognition of a lesion-containing mismatch. A blue line indicates the template strand of replication, and red indicates the newly synthesized strand. (A) MMR recognition of a mismatch containing a lesion on the primer strand will result in degradation of the primer strand followed by resynthesis and elimination of the lesion. (B) MMR recognition of a mismatch containing a lesion on the template strand can result in degradation of the primer strand, but resynthesis will not remove the lesion. (C) MMR recognition of a mismatch containing a lesion outside of replication is problematical. In the absence of a nick in the DNA, there will likely be no DNA excision. However, the appearance of a nick, either spontaneously or by activation of MutL α (see text), could give rise to DNA excision, independent of which strand was newly replicated or contained a lesion.

Fig. 2.

Action of MMR on base-base mispair outside of replication. An oligonucleotide (purple segment) is used to introduce a wild-type sequence into a mutant *TRP5* gene. If the oligonucleotide is not removed by MMR during replication and persists into G2, tryptophan can be made if the oligonucleotide sequence is on the transcribed strand (TS), but not if the oligonucleotide is directed to the nontranscribed strand (NTS). In the presence of MMR, but not in the absence, there are many more Trp+ revertant cells when the oligonucleotide is directed to the NTS, and fewer when the oligonucleotide is directed to the TS [71].

Fig. 3.

MMR activity on an 8-oxoG-A mismatch. 8-oxoGTP is introduced into yeast cells and can be incorporated into the genome; indicated here is 8-oxoGTP incorporated into the NTS of a mutant *TRP5* gene. If the 8-oxoGTP persists in the genome into G2 phase, in the absence of MMR no tryptophan can be produced and no revertant cells appear on selective plates. However, in the presence of MMR, recognition of the 8-oxoG-A mismatch can result in excision of the template strand opposite the 8-oxoG followed by insertion of a C, which allows tryptophan production [71].

Fig. 4. MMR and 8-oxoG replication. (A) In wild-type cells, reversion of the *trp5-A149C* allele is very low. In *ogg1* cells, which do not repair 8-oxoG opposite a C, reversion rates are increased more than 10-fold, indicating that many insertions of A opposite 8-oxoG are tolerated. In the further absence of MMR (*msh6*), reversion rates are increased another order of magnitude, indicating that many A-8-oxoG mispairs were eliminated by MMR [13]. (B) An 8-oxoG was placed into a single location in the yeast genome and its replication monitored [88]. In wild-type cells, replication was 98% accurate. Accuracy was only slightly decreased in the absence of MMR, to 95%. Pol η is the only DNA polymerase that is able to replicate 8-oxoG accurately, and in its absence, but in the presence of MMR, overall accuracy of 8-oxoG replication was 93%. The absence of both MMR and Pol η led to very inaccurate replication, an average of 40%. These experiments suggest that when low levels of 8-oxoG are present in cells, the presence of either Pol η or MMR is sufficient to prevent most mutations, but when higher levels of 8-oxoG are present in the cell (as in *ogg1* cells),

both Pol η and MMR are necessary. Even higher levels of oxidative damage in the cell can outstrip the capacity of Ogg1, Pol η , and MMR to prevent mutation [13].

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