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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.

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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 *Escherichia coli*. Although not incorrect, that 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

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mutations, and more generally in/del mutations, are much greater than for base pair substitutions. There are two mismatch recognition complexes in many eukaryotes, MutSα and MutSβ, and we have recently suggested that the existence of MutSβ 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 5000-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 MutSα 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 Tham et al. 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 knows 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.