The *Escherichia coli* Mismatch Repair Protein MutL Recruits the Vsr and MutH Endonucleases in Response to DNA Damage

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The activities of the Vsr and MutH endonucleases of *Escherichia coli* are stimulated by MutL. The interaction of MutL with each enzyme is enhanced in vivo by 2-aminopurine treatment and by inactivation of the *mutY* gene. We hypothesize that MutL recruits the endonucleases to sites of DNA damage.

The *Escherichia coli* Dcm protein methylates the second C of CCWGG sites (W = A or T). Deamination of 5-methylcytosine converts CG base pairs to T/G mismatches, causing CCWGG-to-CTWGG transition mutations. Very-short-patch (VSP) repair minimizes these mutations (2). Repair is initiated by a sequence- and mismatch-specific endonuclease, Vsr, which cleaves the DNA 5' of the T. DNA polymerase I removes the T along with a few 3' nucleotides and resynthesizes the missing bases, restoring the CG base pair. Vsr is both necessary and sufficient for initiating VSP repair. However, two other proteins, MutS and MutL, enhance VSP repair of deamination damage (1).

MutS and MutL are best known for their roles in postreplication mismatch repair (MMR) (9, 11). MutL couples mismatch recognition by MutS to the activation of MutH, an endonuclease that cleaves the unmethylated strand of GATC sequences that are transiently hemimethylated following DNA replication. The nicked strand, containing the erroneous base, is removed by the UvR helicase and one of several exonucleases to beyond the mismatch and then resynthesized by DNA polymerase III.

MutL stimulates the endonuclease activities of both Vsr and MutH in vitro (8, 17). The requirements for stimulation are the same: a mismatch, MutS, and ATP hydrolysis by MutL (8, 8a). Cross-linking studies showed that MutH and Vsr interact with the same region in the N-terminal domain of MutL (Heinze et al., submitted). Competition of Vsr with MutH for access to MutL explains the ability of Vsr to inactivate MMR in vivo (15). We found no false positives or false negatives. Furthermore, since the assay relies on reconstitution of a soluble protein (adenylate cyclase), the DNA repair proteins are free to interact with the DNA (Fig. 1).

2-Aminopurine (2AP) mispairs with C during DNA replication, causing transition and frameshift mutations (5). The transitions are due primarily to the mismatch itself; the frameshifts are due to saturation of MMR, which leaves slipped-strand intermediates caused by DNA replication errors unrepaired (19). MutS and MutL bind to 2AP/C lesions (22), although the lesions may not be subject to MMR (19). As shown in Fig. 2, treatment with 2AP causes a dose-dependent increase in the interaction of MutL with both Vsr and MutH; dimerization of MutL and interaction of MutL with MutS are somewhat increased.

The MutY adenine glycosylase removes A's which have mispaired with oxidized guanine (8-oxoG) during DNA replication. Cells with a deletion of *mutY* have an elevated frequency of CG-to-AT transversion mutations (18); these are reduced by excess MutS, suggesting that 8-oxoG/A mismatches are also subject to MMR (23). As shown in Fig. 3, the interactions between Vsr and MutL and between MutH and MutL increase in a *mutY* cell (stippled bars). Other interactions, such as MutS dimerization, are unaffected (not shown).

8-OxoG/A mismatches also arise by incorporation of oxidized dGTP opposite A during DNA replication. The MutT nuclease minimizes this by removing oxidized dGTP from the nucleotide pool. The high frequency of AT-to-CG mutations in *mutT* strains is unaffected by the status of the MMR system (7, 21, 23), possibly because these 8-oxoG/A mispairs are in a configuration that MutS does not recognize. As shown in Fig. 3, neither the interaction between MutL and Vsr nor that between MutL and MutH is elevated in a *mutT* strain (solid bars).

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These data show that mismatches which attract MutS and MutL increase the interaction of MutL with MutH in vivo. Although these mismatches are not subject to VSP repair, they also increase the interaction between MutL and Vsr. The simplest interpretation is that a MutS-MutL complex recruits MutH and Vsr to the DNA independent of the identity of the mismatch. MutS and MutL could then clear the mismatch, delivering the (activated) endonuclease to its specific target site, no matter how far away it is.
Interaction of MutL with MutH, leading to MMR, is probably the default option. However, the MutS-MutL complex may recruit other repair proteins, such as Vsr or UvrB (20), to lesions that are poorly processed by MMR. The T/G mismatch in hemimethylated CTWGG sequences may be one such site. Vsr is expressed at very low levels in growing cells (14), so this recruitment would enhance VSP repair. However, recruitment of Vsr to other lesions would reduce VSP repair. For example, recruitment of Vsr by MutL to 2AP/C lesions (Fig. 2) could explain why CCWGG sites are hotspots for 2AP-induced mutations (4, 19).

We have argued that Vsr is kept at low levels while DNA is replicating to avoid interference with MMR (14). However, if, as we suggest here, MutS and MutL are needed to recruit scarce Vsr to its target sequence, this argument loses its merit. It seems more likely that Vsr levels are kept low to avoid recruitment of Vsr by MutL to 2AP/C lesions (Fig. 2) could explain why CCWGG sites are hotspots for 2AP-induced mutations (4, 19).

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