Mismatch repair proteins as sensors of alkylation DNA damage

The DNA mismatch repair (MMR) system maintains genome integrity by correcting replication errors. MMR also stimulates checkpoint and cell death responses to DNA damage suggested by the resistance of MMR-defective tumor cells to several chemotherapeutic agents. MMR-dependent cytotoxic response may result from futile repair; however, MMR-mediated apoptosis has been genetically separated from its repair function. In a recent issue of *Molecular Cell*, Yoshioka and coworkers show that MMR complexes (MutSα and MutLα) are required for the recruitment of ATR-ATRIP to sites of alkylation damage, demonstrating that MMR complexes can function as sensors in DNA damage signal transduction.

The DNA mismatch repair (MMR) pathway plays a critical role in the maintenance of genome integrity. The evolutionarily conserved MMR pathway functions in postreplicative genome surveillance by repairing base substitution mutations as well as small insertions/deletions (IDLs) caused by replication errors. In mammalian cells, MMR is initiated by MSH2-containing heterodimeric complexes of MSH2-MSH6 (MutSα) and MSH2-MSH3 (MutSβ). The MutSα complex recognizes base-base mispairs and single base IDLs, while the MutSβ complex detects larger IDLs to initiate the repair process. The MutSα and MutSβ complexes undergo ATP-hydrolysis-dependent conformational transitions following binding to DNA mispairs or IDLs to recruit a heterodimeric complex of MLH1 and PMS2 (MutLα). This interaction between the MutS and the MutL complexes is essential for the activation of subsequent MMR steps, i.e., the excision of the mispairs and IDLs and the resynthesis of the DNA strand (Buermeyer et al., 1999; Kolodner and Marsischky, 1999; Kunkel and Erie, 2005; Iyer et al., 2006). Hereditary mutations in *MSH2* and *MLH1* predispose humans to colon and endometrium cancers, and mice to intestinal cancer and lymphoma. Epigenetic silencing of *MLH1* has also been detected in a wide variety of sporadic human cancers. Thus, MMR is an important tumor suppression mechanism in mammals.

In multicellular organisms, DNA repair is complemented by the active elimination of damaged cells through apoptosis, necrosis, mitotic catastrophe, or the induction of premature senescence, to suppress tumor development. These damage-induced tumor suppression pathways are coordinately regulated by a signaling network consisting of damage sensors, adaptors, mediators, and protein kinases (Wang and Cho, 2004). Over the past decade, evidence has accumulated to suggest that MMR not only repairs DNA but also stimulates DNA damage-induced G2 checkpoint and apoptosis (Iyer et al., 2006; Fishel, 2001; Stojic et al., 2004). While MMR-proficient cells respond to cisplatin, 6-thioguanine (6-TG), and S_N1 DNA methylators such as temozolomide and MNNG (N-methyl-N′-nitro-N-nitrosoguanidine) by undergoing G2 arrest followed by programmed cell death, MMR-deficient cells are defective in this response, resulting in an increased resistance to the genotoxic effects of DNA damage-inducing agents. Because cisplatin and DNA alkylating agents are used in cancer therapy, there is considerable interest in understanding how MMR-deficient cancer cells acquire resistance to these drugs.

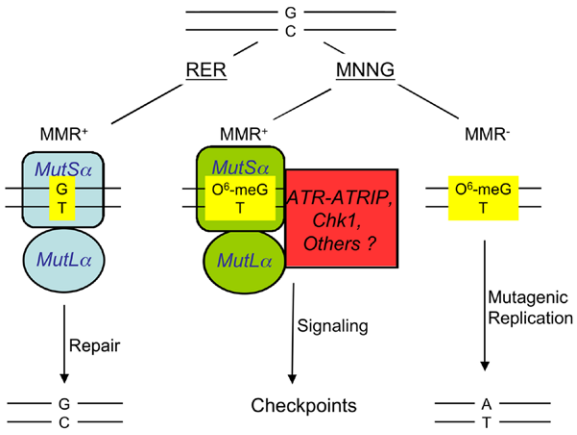

Figure 1. Signaling function of MMR proteins

Replication errors (RER) or exposure to MNNG at G/C base pairs can result in G/T or O⁶-meG/T mispairs, respectively. In MMR-proficient cells (MMR⁺), MutSα and MutLα signal repair of G/T mispairs to restore the G/C base pair (left). As demonstrated by Yoshioka et al. (2006), MutSα and MutLα also bind specifically to sites of O⁶-meG/T mispairs in DNA and recruit ATR-ATRIP. The recruitment of ATR-ATRIP only occurs in the presence of MutSα and MutLα, leading to the phosphorylation of Chk1 and other downstream targets of ATR kinase to activate cell cycle checkpoints (middle). MMR-deficient cells (MMR⁻) escape checkpoint signaling and accumulate G:C>A:T mutations at O⁶-meG/T sites during following rounds of replication (right).

Two opposing models have been proposed to account for the MMR-dependent activation of the G2 checkpoint and programmed cell death—the futile cycle model and the direct signaling model.

The futile cycle model

The futile cycle model considers the MMR pathway to have a single function, i.e., DNA repair. The MMR-dependent activation of G2 checkpoint or programmed cell death is due to the creation of DNA strand breaks that result from a futile attempt of the MMR pathway to repair alkylated DNA (Karran, 2001). Exposure to S_N1 alkylating agents such as MNNG creates O⁶-methylguanine (O⁶-meG), which can pair with cytosine (C) or thymidine (T). Replication of a template strand containing O⁶-methylguanine would lead to the formation of O⁶-meG/C or O⁶-meG/T. In MMR-proficient cells, O⁶-meG/C or O⁶-meG/T are recognized by MutSα, which interacts with MutLα to initiate excision of the newly synthesized strand. Because O⁶-meG is in the template strand, which is not excised by MMR, abortive repair cycles would ensue to cause DNA strand breaks and checkpoint activation. In MMR-deficient cells, O⁶-meG/C or O⁶-meG/T are not processed, and therefore these cells survive and display increased mutagenesis.

The direct signaling model

The direct signaling model proposes that MMR complexes have two distinct functions, one in repair and the other in DNA damage signal transduction (Kat et al., 1993; Fishel, 2001). The MutSα complex, by virtue of its recognition of O⁶-meG/C, O⁶-meG/T or platinated DNA, can function as a sensor to activate the DNA damage-signaling network. Genetic evidence for this model was provided with the creation of "separation of function" mutants in the murine *Msh2* and *Msh6* genes (Lin et al., 2004; Yang et al., 2004). In these

studies, mouse lines were engineered to carry missense mutations in Msh2 and Msh6, located within the ATP binding site or in close proximity to it. These mutations disrupted the ATP processing ability of MutSα, which is an essential function for DNA repair in both prokaryotes and eukaryotes. Biochemical analysis of cell extracts from these mouse lines showed that, although the mutant MutSα complexes had normal affinity to mismatched base pairs, the defect in ATP processing prevented the downstream steps of DNA repair. Nevertheless, the mutant MutSα conferred a normal apoptotic response to MNNG, cisplatin, and other agents, demonstrating that DNA damage signaling by MMR proteins can occur independently of normal strand excision. These studies show that the repair function of MMR is dispensable for the MMR-dependent apoptotic response to DNA damage.

The report by Yoshioka et al. (2006) in a recent issue of *Molecular Cell* provides further evidence for the idea that MutSα and MutLα participate directly in the activation of ATR by O^6-meG/T mispairs. The authors demonstrate an MMR-dependent and S phase-specific activation of ATR, as measured by the phosphorylation of Chk1 and SMC1 (structure maintenance of chromosome 1), in MNNG-treated HeLa cells. This response is not observed in MSH2- or MLH1-deficient cells, suggesting that MMR recognition of O^6-meG/T or O^6-meG/C may be involved in ATR activation. The authors then showed that MutSα bound to O^6-meG/C in a nonspecific manner, but it bound to O^6-meG/T with a high affinity comparable to its binding to a G/T mispair, although the affinity of MutSα for O^6-meG/T varied depending on the sequence context. When nuclear extracts were incubated with plasmids bearing O^6-meG/T mispairs, ATR and its associated adaptor protein, ATRIP, were found to be preferentially crosslinked to the site of O^6-meG/T, but not to the G/T mispairs (Figure 1). The MutSα and MutLα complexes are also preferentially crosslinked to plasmid sites bearing the O^6-meG/T mispairs, although these MMR complexes also preferentially crosslinked to the G/T mispairs (Figure 1). Furthermore, a DNA-protein complex assembled with the O^6-meG/T mispair-bearing plasmid and nuclear extract phosphorylated Chk1, whereas the G/T mispair-bearing DNA-protein complex did not contain detectable Chk1-kinase activity. The O^6-meG/T-bearing DNA used in these studies was not subjected to nucleolytic degradation, indicating that ATR-ATRIP recruitment occurs independently of MMR-mediated excision repair.

This latest evidence in support of MMR complexes as damage sensors has raised new questions regarding DNA damage signal transduction. The direct signaling model would predict that a regulatory mechanism exists to direct the MMR complexes toward either DNA excison/resynthesis or the ATR-ATRIP pathways (Figure 1). The work of Yoshioka et al. suggests that regulation would be dependent on the DNA ligand, as the G/T mispair does not recruit ATR-ATRIP (but it should recruit repair enzymes), whereas the O^6-meG/T mispair recruits ATR-ATRIP (it is unclear whether it also recruits repair enzymes). The ATR-ATRIP complex has been shown to bind RPA-coated single-stranded (ss) DNA (Zou and Elledge, 2003). ATR has also been shown to physically interact with MSH2 (Wang and Qin, 2003). The study of Yoshioka et al. demonstrates that the recruitment of ATR-ATRIP to the O^6-meG/T mispair requires MutSα and MutLα; however, they did not observe a preferential crosslinking of RPA to the O^6-meG/T site. It is conceivable that MutSα and MutLα may recruit ATR-ATRIP independently of RPA-ssDNA, or alternatively, a limited RPA-ssDNA region might be created at the O^6-meG/T mispair to allow the binding of ATR-ATRIP. The observation that ATR-ATRIP is not recruited to G/T mispairs despite the binding of MutSα and MutLα at these sites indicates that MMR-dependent assembly of the damage-signaling complex requires additional factors. Identification of those factors is within reach with the DNA-protein complex assembly methods devised by Yoshioka et al. Further dissection of the MMR-dependent signaling complex would hopefully lead to a resolution of the two opposing models on the role of MMR in DNA damage response.

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