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Endonuclease activities of MutLα and its homologs in DNA mismatch repair

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ABSTRACT

MutLα is a key component of the DNA mismatch repair system in eukaryotes. The DNA mismatch repair system has several genetic stabilization functions. Of these functions, DNA mismatch repair is the major one. The loss of MutLα abolishes DNA mismatch repair, thereby predisposing humans to cancer. MutLα has an endonuclease activity that is required for DNA mismatch repair. The endonuclease activity of MutLα depends on the $DQHA(X)_2E(X)_4E$ motif which is a part of the active site of the nuclease. This motif is also present in many bacterial MutL and eukaryotic MutLγ proteins, DNA mismatch repair system factors that are homologous to MutLα. Recent studies have shown that yeast MutLγ and several MutL proteins containing the $DQHA(X)_2E(X)_4E$ motif possess endonuclease activities. Here, we review the endonuclease activities of MutLα and its homologs in the context of DNA mismatch repair.

1. Introduction

The DNA mismatch repair (MMR) system genes have been found in the majority of living organisms, indicating that this DNA repair system is important for maintaining life. Studies in model organisms and human cells have demonstrated that the MMR system has multiple functions in DNA metabolism ((1-16), and other reviews in this special issue). Most functions of the MMR system promote genome stability, but some of its functions contribute to the instability

of certain genomic loci (7,17,18). Repair of DNA mismatches that are formed during replication and homologous recombination is the major genetic stabilization function of the MMR system (19-25). MMR is more efficient on the lagging strand than on the leading strand (26). The most common substrates for the MMR system are small DNA insertions/deletions and single DNA base-base mispairs (27-29). The MMR system also corrects DNA mispairs containing 8-oxoguanine and other oxidatively damaged bases (17,30-33). Furthermore, the MMR system removes 1-nucleotide Okazaki fragment flaps (34) and single ribonucleotides, which are incorporated into DNA opposite noncomplementary deoxyribonucleotides (35). MutSα (MSH2-MSH6 heterodimer) and MutLα (MLH1-PMS2 heterodimer in humans and MLH1-PMS1 heterodimer in the yeast *S. cerevisiae*) are required for the majority of MMR events in eukaryotes (28,36,37). MutSα is the key mismatch recognition factor (28,29,38), and MutLα acts as an endonuclease in MMR (39,40). In addition to MutSα and MutLα, MutSβ (MSH2-MSH3 heterodimer) (29,41-45), Exonuclease 1 (EXO1) (45-48), proliferating cell nuclear antigen (PCNA) (49-53), replication factor C (RFC) (53), replication protein A (RPA) (52,54,55), DNA polymerase δ (Pol δ) (44,51,56-58), MutLγ (MLH1-MLH3 heterodimer) (59-61), the 3′→5′ exonuclease activity of Pol δ (62), HMGB1 (44,63,64), DNA ligase I (44), and RNAse H2 (65,66) have also been implicated in eukaryotic MMR. Furthermore, PARP1 (67), CAF-1-dependent chromatin assembly (68,69), and SETD2-dependent histone H3 trimethylation on K36 (16,70) have been suggested to regulate MMR. Genetic inactivation of MMR strongly predisposes humans and mice to several types of cancer (43,71-82).

A key feature of MMR is its strand specificity that ensures that a mismatch is corrected on the daughter strand, but not the parental strand. Without strand specificity MMR would be a mutagenic process because it would often result in the removal of a mismatch on the parental strand, converting the replication error into a mutation. MMR is directed to the daughter strands by strand discrimination signals. Strong evidence indicates that strand breaks involved in the

leading- and lagging-strand synthesis are the strand discrimination signals for eukaryotic MMR. First, eukaryotic MMR in nuclear extracts, whole-cell extracts, and reconstituted systems occurs on the discontinuous strands, but not the continuous strands (39,40,44,45,48,52,53,57,58,83-86). Second, strand breaks produced by RNAse H2 serve as strand discrimination signals for a small but significant subset of MMR events on the leading strand in the yeast *S. cerevisiae* (65,66). Here we review how the endonuclease activity of MutL homologs is involved in creating strand breaks during MMR, and how these strand breaks are directed to the daughter strand via interactions with other components of the MMR machinery.

2. Endonuclease activity of MutLα

MutLα is essential for MMR and many other functions of the MMR system (36,37,73,76,77,87-90). During MMR, the major mismatch recognition factor MutSα, the replicative clamp PCNA, the clamp loader RFC, and ATP-Mg^{2+} activate MutLα endonuclease to incise the discontinuous daughter strand near the mismatch (39,40,58). The second mismatch recognition factor MutSβ can substitute for MutSα in the activation of the endonuclease provided that the mismatch is a small insertion/deletion loop (91). The function of RFC in the endonuclease activation is to load PCNA at a strand discontinuity (92). The incision of the discontinuous daughter strand by MutLα initiates downstream reactions that are necessary to remove the mismatch (39,40,58).

MutLα is also able to act as an ATP-Mn^{2+}-dependent endonuclease in defined reactions that are not involved in MMR (39,40). In these reactions, MutLα alone nicks DNA. RFC and PCNA strongly stimulate the ATP-Mn^{2+}-dependent endonuclease activity of MutLα. The ATP-Mn^{2+}-dependent endonuclease activity of MutLα is maximal in the presence of 1 mM Mn^{2+}, but is not detectable at physiological Mn^{2+} concentration (35 μM (93)). The latter observation suggests that the ATP-Mn^{2+}-dependent endonuclease activity of MutLα is silent *in vivo* and

does not contribute to eukaryotic MMR (39). In agreement with this idea, Mn^{2+} is not required for the endonucleolytic function of MutLα in reconstituted MMR reactions (39,40,53,57,58).

The C-terminal part of the PMS2 subunit of hMutLα endonuclease hosts a metal-binding site that includes the DQHA$(X)_2$E$(X)_4$E motif at position 699-710 (39,94,95). A hMutLα variant carrying the PMS2 D699N substitution (hMutLα-D699N) and another one carrying the PMS2 E705K substitution (hMutLα-E705K) are unable to act as endonucleases in MMR in nuclear extracts and reconstituted systems (39,58). The hMutLα-D699N and hMutLα-E705K mutant proteins also lack the ATP-Mn^{2+}-dependent endonuclease and metal-binding activities. Consistent with these biochemical findings, *PMS2-E705K* expression in PMS2-deficient cells does not rescue their defect in MMR and the MMR system-dependent apoptotic response to an S_{N1}-type methylating drug (89,96). Further biochemical examination of the DQHA$(X)_2$E$(X)_4$E motif has suggested that the PMS2 H701 is required for the endonucleolytic function of MutLα in MMR, but the PMS2 E710 is not (97). If the PMS2 E710 is indeed not needed for the action of MutLα endonuclease in MMR, its conservation suggests that it may be involved in another as yet undefined function of MutLα..

The Pms1 subunit of yMutLα and the homologous PMS2 subunit of mMutLα also contain the DQHA$(X)_2$E$(X)_4$E motif (39). The yPMS1 E707 and mPMS2 E702 are located at the same position within the DQHA$(X)_2$E$(X)_4$E motif as the hPMS2 E705. Not only does the yPMS1 E707K substitution disrupt the endonuclease activity of yMutLα (40), it also completely inactivates MMR and strongly compromises the MMR system-dependent suppression of homeologous recombination (40,89,96). In agreement with these findings, the mouse *Pms2*$^{E702K/E702K}$ mutation causes genetic instability, MMR deficiency, and strong predisposition to cancer (79). Importantly, the phenotypes of the mouse *Pms2*$^{E702K/E702K}$ mutation are the same or nearly the same as those of the *Pms2*$^{-/-}$. Collectively, the studies in the human, yeast, and mouse systems have provided strong evidence that the endonuclease activity of MutLα is

required for multiple functions of the MMR system: MMR, cancer suppression, prevention of homeologous recombination, and initiation of the apoptotic response to specific DNA lesions.

The inactivation of the metal-binding and endonuclease activities of hMutLα by the PMS2 D699N and E705K substitutions led to the suggestion that the $DQHA(X)_2E(X)_4E$ motif is part of the endonuclease active site (39,40). The structural studies of *B. subtilis* MutL (98) and yeast MutLα (99) have confirmed this idea (94,95). In the structural model of the C-terminal domain of *B. subtilis* MutL, the $DQHA(X)_2E(X)_4E$ motif and three other conserved motifs (ACR, CP/NHGRP, and FXR (97)) form an endonuclease active site (98). Though this active site differs from active sites of nucleases from other families, it has features that characterize many endonuclease active sites: a highly conserved aspartate residue and the ability to bind two divalent metal ions (100). Modeling of DNA onto the structural model of the *B. subtilis* MutL domain places the $DQHA(X)_2E(X)_4E$ motif and a phosphodiester bond in the DNA within a distance from each other that allows the carboxylate side chain of the aspartate residue in the first position of the motif to activate catalysis of an endonucleolytic reaction (98). yPMS1 amino acid residues located within the $DQHA(X)_2E(X)_4E$, ACR, and CP/NHGRP motifs and the yMLH1 C769 constitute the yMutLα endonuclease active site (99). The yMutLα endonuclease active site contains two Zn^{2+} ions (99). Two Zn^{2+} ions have also been detected in the endonuclease active site of *B. subtilis* MutL (98). The first glutamate residue of the $DQHA(X)_2E(X)_4E$ motif in both yMutLα and *B. subtilis* MutL participates in coordination of the two Zn^{2+} ions. The Zn^{2+} ions have been proposed to be important for regulation of the endonuclease activities of these proteins (97-99). Despite the significant progress that has already been achieved, more research is needed to elucidate how MutLα and its homologs accomplish and regulate the endonucleolytic reaction.

The N-terminal parts of MutLα endonuclease subunits carry conserved ATPase domains that are also present in bacterial MutLs and other members of the GHKL superfamily of proteins

(101-105). Accordingly, each subunit of MutLα binds and hydrolyzes ATP. ATP binding and hydrolysis by MutLα subunits drive large conformational changes of the protein (106,107). As described below, the effects of ATP binding and hydrolysis by each MutLα subunit on MMR have been investigated in detail (106,108-110). The yMLH1 N35 and yPMS1 N65 are required for ATP binding by the respective subunits (103,104,106,108). The catalytic residue for ATP hydrolysis by yMLH1 is the E31 and equivalent residue in yPMS1 is the E61 (103,104,106,108). The disruption of ATP-binding activity of either yMLH1 with the N35A or yPMS1 with the N65A completely inactivates yeast MMR (108). On the other hand, the elimination of the ATP hydrolytic activity of yMLH1 with the E31A or yPMS1 with the E61A results only in a weak defect in yeast MMR.

Amino acid residues essential for ATP binding by hMLH1 are the N38 and D63, and the functionally similar residues in hPMS2 are the N45 and D70 (103,104,109,110). Blocking ATP binding with the hMLH1 N38A, hPMS2 N45A, or hPMS2 D70N substitution abolishes human MMR (109). The catalytic residues for hMLH1 and hPMS2 ATPases are the E34 and E41, respectively (103,104,109,110). The hMLH1 E34A and hPMS2 E41A substitutions each cause only a weak defect in human MMR. However, the hMLH1-E34A hPMS2-E41A double substitution obliterates human MMR (109) by inactivating the ability of MutLα to incise the discontinuous strand in the presence of MutSα, PCNA, RFC, ATP-Mg^{2+}, and a mismatch (39). Collectively, these findings support the following conclusions. First, the endonucleolytic function of MutLα in MMR is abolished when either of its subunits loses the ability to bind ATP or both subunits are unable to hydrolyze ATP. Second, the endonucleolytic function of MutLα in MMR is only weakly compromised when only one of its subunits is defective in ATP hydrolysis.

MutLα binds both single-stranded and double-stranded DNAs (105,111,112). A structure-based candidate approach has revealed that the yMLH1-R273E-R274E double substitution, which weakens the DNA-binding activity of yMutLα, completely inactivates yeast

MMR (112). The result supports the view that the ability of MutLα to bind DNA is required for the endonucleolytic function of this protein in MMR.

3. MutLα and human nick-directed MMR

3′- and 5′-nick directed modes of MMR have been demonstrated in extracts of human, Drosophila, mouse, and Xenopus cells and reconstituted systems (44,45,57,58,83,84,113-115). (3′-nick directed MMR occurs on a 3′ heteroduplex DNA, which contains a nick 3′ to a mismatch, and 5′-nick directed MMR takes place on a 5′ heteroduplex DNA, which carries a nick 5′ to a mismatch.) Mammalian MutLα, MutSα, MutSβ, EXO1, PCNA, RFC, and RPA are involved in both 3′- and 5′-nick directed MMR in extracts and reconstituted systems (28,29,37,39,41,44,46,48,52-54,57,58,91,116,117). The establishment and analysis of the reconstituted systems has been instrumental for defining the functions of these proteins in nick-directed MMR (39,44,52,53,57,58,91). The reconstituted systems rely on the action of MutSα or MutSβ for initiation and progression of the reaction. MutSα is the primary mismatch recognition factor required for repair of base-base and 1-nt insertion/deletion mismatches (28). In addition, MutSα is needed for repair of a large fraction of 2-12-nt insertion/deletion mismatches (29). Repair of the remainder of 2-12-nt insertion/deletion mismatches depends on MutSβ (29). The simplest reconstituted system includes MutSα, MutLα, EXO1, and RPA, and performs a 5′-nick directed mismatch excision (52). MutLα endonuclease activity is silent during the reconstituted 5′-nick directed mismatch excision due to the absence of loaded PCNA. The reconstituted 5′-nick directed mismatch excision is initiated by the recognition of a base-base mispair by MutSα. Upon mismatch recognition, MutSα activates EXO1 to degrade a mismatch-containing segment of the discontinuous strand in a 5′→3′ excision reaction that initiates from a pre-existing nick and is stimulated by RPA (52). Once the mismatch is excised, MutSα suppresses EXO1 activity protecting the DNA from unnecessary degradation (52). Though MutLα does not influence the

excision on heteroduplex DNA, the protein significantly enhances MutSα-dependent suppression of the exonucleolytic degradation on homoduplex DNA. The effect is probably a result of the inhibition of EXO1 activity by MutLα (118) and the MutSα-MutLα complex (119,120).

The addition of PCNA and its loader RFC to the four-protein system produces a six-protein system that is proficient in both 5′- and 3′-nick directed excision of base-base mismatches (53). Both modes of excision occurring in this system depend on MutSα and the 5′→3′-directed exonuclease activity of EXO1 (53,121). Furthermore, 3′-nick directed mismatch excision requires the endonuclease activity of MutLα, but 5′-nick directed mismatch excision does not. During 3′-nick directed mismatch excision, MutLα endonuclease activated by MutSα and loaded PCNA incises the discontinuous strand of 3′ heteroduplex DNA producing strand breaks that are often 5′ to the mismatch (39). A 5′ strand break produced by MutLα is used by EXO1 as the starting point of 5′→3′ excision that removes a part of the discontinuous strand containing the mismatch (**Fig. 1**). The effect of RFC on the 3′ excision is twofold. First, it loads PCNA onto the 3′ heteroduplex DNA. Second, RFC inhibits EXO1-mediated 5′→3′ excision that initiates from the pre-existing 3′ nick and occurs in a direction opposite to the location of the mismatch. RFC activity responsible for this effect has been mapped to the N-terminal domain of the largest subunit of the protein. Supplementation of the six-protein system with Pol δ yields a system that is competent in both 5′- and 3′-nick directed MMR (57). The reconstituted 3′-nick directed MMR depends on all seven proteins, but the reconstituted 5′-nick directed MMR does not require MutLα endonuclease activity.

Contrary to the reconstituted 5′-nick directed MMR (57), 5′-nick directed MMR in some but not all nuclear extracts involves MutLα (37,122,123). We hypothesize that 5′-nick directed MMR *in vivo* requires the endonuclease activity of MutLα (**Fig. 1**). This hypothesis is supported by the observations that MutLα deficiency causes the same mutator phenotype and cancer

predisposition as MutSα deficiency (26,50,90,124). It is probable that some unknown protein factors that are absent in the reconstituted system (57) do not allow 5′-nick directed MMR to occur in a MutLα-independent manner *in vivo*.

Loss of *EXO1* in yeast and mouse cells causes weak or modest defects in MMR (47,62,125). Moreover, inactivation of *EXO1* has not been linked to carcinogenesis in humans, and *Exo1*$^{-/-}$ mice display only a moderate predisposition to cancer (125). These observations have suggested that MMR remains functional in the absence of EXO1. As described above, MutLα endonuclease activity plays a central role in MMR that involves EXO1 (**Fig. 1**). In addition, MutLα is essential for EXO1-independent MMR (51,126,127). Analysis of whole-cell extracts and reconstituted systems has identified a mechanism for EXO1-independent MMR that does not involve mismatch excision (58). The mechanism requires the activities of MutSα, MutLα, PCNA, RFC, and Pol δ, and is directed by a strand discontinuity. In this mechanism, the activated MutLα endonuclease cleaves the discontinuous strand of a heteroduplex DNA producing strand breaks near the mismatch (**Fig. 1**). A new 3′ end created by the MutLα cleavage 5′ to the mismatch primes DNA synthesis that displaces a part of the discontinuous strand containing the mismatch. The strand-displacement synthesis is carried out by Pol δ holoenzyme and strongly stimulated by RPA. Thus, the combined action of six human proteins corrects mismatches in an excision-free process. Time-course analysis of the EXO1-independent repair has indicated that its rate is significantly slower than that of the repair involving EXO1 (58). This suggests that in the presence of EXO1, MMR probably occurs via the EXO1-dependent mechanism (**Fig. 1**). The involvement of the strand-displacement mechanism in yeast EXO1-independent MMR is supported by studies that have demonstrated that ablation of the Pol32 subunit causes defects in both the strand-displacement activity of Pol δ and MMR (51,128,129). A recent report has described that an MSH6 mutant defective in the interaction with PCNA does not support EXO1-independent MMR in yeast cells (130). It will be interesting

to determine whether this mutation affects the reconstituted strand displacement-based MMR. The strand-displacement mechanism is probably not the only option for EXO1-independent MMR (62). Given the importance of EXO1-independent MMR for the suppression of carcinogenesis, it is important to continue to investigate its key players and mechanisms.

4. Models for the activation of MutLα endonuclease in MMR

Despite the importance of the endonuclease activity of MutLα for MMR (39,40,79), the mechanism of its activation in this process remains undefined. Two models that are not mutually exclusive outline possible mechanisms of the MutSα-, PCNA-, RFC-, ATP-, and mismatch-dependent activation of MutLα endonuclease in MMR (**Fig. 2**). The models are based on the observations that 1) during MMR, MutSα, PCNA, RFC, and ATP-Mg^{2+} activate MutLα to incise the discontinuous strand (39,40,92), 2) the ATP-Mn^{2+}-dependent endonuclease activity of MutLα incises both strands of nicked heteroduplex DNA with the same efficiency (40), 3) PCNA and RFC are sufficient to direct the ATP-Mn^{2+}-dependent endonuclease activity of MutLα to cut the discontinuous strand (40), 4) MutLα activated by MutSα, PCNA, and RFC on heteroduplex DNA lacking a pre-existing strand break weakly incises both strands without displaying a strand bias (92), 5) MutLα and MutSα form a complex on heteroduplex DNA in a mismatch-dependent manner (119,120), 6) RFC function in the activation of MutLα endonuclease is to load PCNA (92), 7) after loading PCNA, RFC dissociates from DNA (131), and 8) when loaded at a strand discontinuity by RFC, the PCNA trimer is oriented such that its face containing the hydrophobic pocket, required for the interactions with numerous DNA replication and repair proteins (132-134), is directed towards the strand discontinuity (135).

In one of the models (**Fig. 2**, left panel), assembly of the complex between MutLα and loaded PCNA at a strand break is an early event in the activation of the endonuclease. The complex formation depends on the presence of the hydrophobic pocket in PCNA. During,

immediately before, or after the complex formation, the MutLα utilizes the strand break to recognize which of the strands is discontinuous. A transient or permanent kink at the strand break (136) and/or the loaded PCNA might facilitate the recognition of the discontinuous strand by the MutLα. The strand recognition triggers ATP-dependent adoption of activated conformation by the MutLα. In the activated conformation, the MutLα is ready to incise the discontinuous strand, but not the continuous strand. Once the MutLα-PCNA complex is formed it starts sliding along DNA. When the sliding MutLα-PCNA complex contacts a mismatch-activated MutSα (137,138), the mismatch recognition factor activates the MutLα to incise the discontinuous strand.

The other model envisions that the formation of the complex between mismatch-activated MutSα and MutLα on heteroduplex DNA occurs before loaded PCNA is engaged in the activation of the nuclease (**Fig. 2**, right panel). After the MutSα-MutLα complex is formed, it slides on the DNA and contacts loaded PCNA. This results in assembly of MutSα-MutLα-PCNA complex. When the three-protein complex sliding along the DNA reaches a strand-break, the MutLα recognizes the discontinuous strand and assumes the activated conformation. The complex resumes its movement on the DNA and then the activated MutLα incises the discontinuous strand. Though the two models are consistent with the existing data, it will be important to determine the actual mechanism of MutLα activation in both MutSα- and MutSβ-dependent MMR. The mechanism of MutLα endonuclease activation in MutSβ-dependent MMR is probably different from that in MutSα-dependent MMR (91).

5. Endonuclease activity of prokaryotic and eukaryotic MutLα homologs

MMR in bacteria depends on homodimeric MutL proteins that are prokaryotic homologs of MutLα (98,139-142). Many MutLs contain the DQHA$(X)_2$E$(X)_4$E motif (39,40,97) or a slightly modified sequence (142,143). Up to date, endonuclease activity has been demonstrated for the

following DQHA$(X)_2E(X)_4E$ motif-containing MutLs: *A. aeolicus* MutL (141,143-145), *T. thermophilus* MutL (141), *B. subtilis* MutL (98), *P. aeruginosa* MutL (142,146), and *N. gonorrhoeae* MutL (147,148). These enzymes have Mn^{2+}-dependent activities that nick supercoiled DNA. ngMutL and paMutL endonucleases are also activated by Mg^{2+}(142,147). Endonuclease activities of these proteins are stimulated or inhibited by ATP (98,141,142,144,147,149). The involvement of endonuclease activities of ttMutL, bsMutL, and paMutL in bacterial MMR has been investigated (98,141,142). Replacing the aspartate residue of the DQHA$(X)_2E(X)_4E$ motif with an asparagine residue in ttMutL and bsMutL eliminates their Mn^{2+}-dependent endonuclease activities and the function of these proteins in MMR (98,141). A different result has been obtained during a similar biochemical and genetic examination of paMutL endonuclease (142). The study has revealed that the D-to-N substitution in the DQHA$(X)_2E(X)_4E$ motif of paMutL does not affect the endonuclease activity of this MutLα homolog, but abolishes MMR in *P. aeruginosa*. Thus, the studies of ttMutL and bsMutL (98,141) support the conclusion that MutL proteins containing the DQHA$(X)_2E(X)_4E$ motif act as endonucleases in MMR, but the analysis of paMutL (142) contradicts this conclusion. Given that the endonuclease activity of the mutant paMutL was measured in the reactions that are not provoked by a mismatch, it is possible that elimination of paMutL endonuclease activity by the D-N substitution can only be observed in *P. aeruginosa* mismatch-provoked reactions. It will be important to address this possibility in future studies.

The effects of protein factors implicated in MMR on the endonuclease activity of one MutL protein, ttMutL, have been determined (149). The endonuclease activity of ttMutL that nicks supercoiled DNA is strongly stimulated by ttMutS, a mismatch, ATP, and Mn^{2+} (149). Replacing Mn^{2+} with Mg^{2+} abolishes the endonuclease activation. The presence of the clamp and a clamp loader variant does not influence the Mn^{2+}-dependent endonuclease activity of ttMutL even when the heteroduplex DNA carries a strand break. These findings have indicated the ttMutS- and mismatch-dependent activation of ttMutL does not require Mg^{2+}, the clamp, the

clamp loader, and a pre-existing strand break. Therefore, the mechanism of MMR in *T. thermophilus* may be quite different from that in human cells.

MutLγ is a eukaryotic MutLα homolog (59,150) that has the DQHA$(X)_2$E$(X)_4$E motif in its MLH3 subunit (39). yMutLγ has an ATP-independent nicking activity that is activated by Mg^{2+}, Mn^{2+}, or Co^{2+} ions (151,152). yMutLγ endonuclease plays a role in yeast MMR, acting in the MutSβ-dependent pathway (59,60,151,152). The D-to-N substitution in the DQHA$(X)_2$E$(X)_4$E motif eliminates the impact of yMutLγ on MMR (60). Strikingly, the endonuclease activity of yMutLγ is not affected by yRFC and yPCNA (151,152), but stimulated by yMutSβ (152). These results suggest that MutLγ endonuclease promotes MMR through a mechanism that differs from the one involving the MutLα endonuclease.

In summary, recent biochemical, genetic, and structural studies have provided novel insights into the functions of MutLα and some of its homologs. Future studies will undoubtedly lead to new discoveries that will advance our understanding of human MutLα endonuclease and its contribution to MMR, cancer suppression, and other functions of the MMR system.

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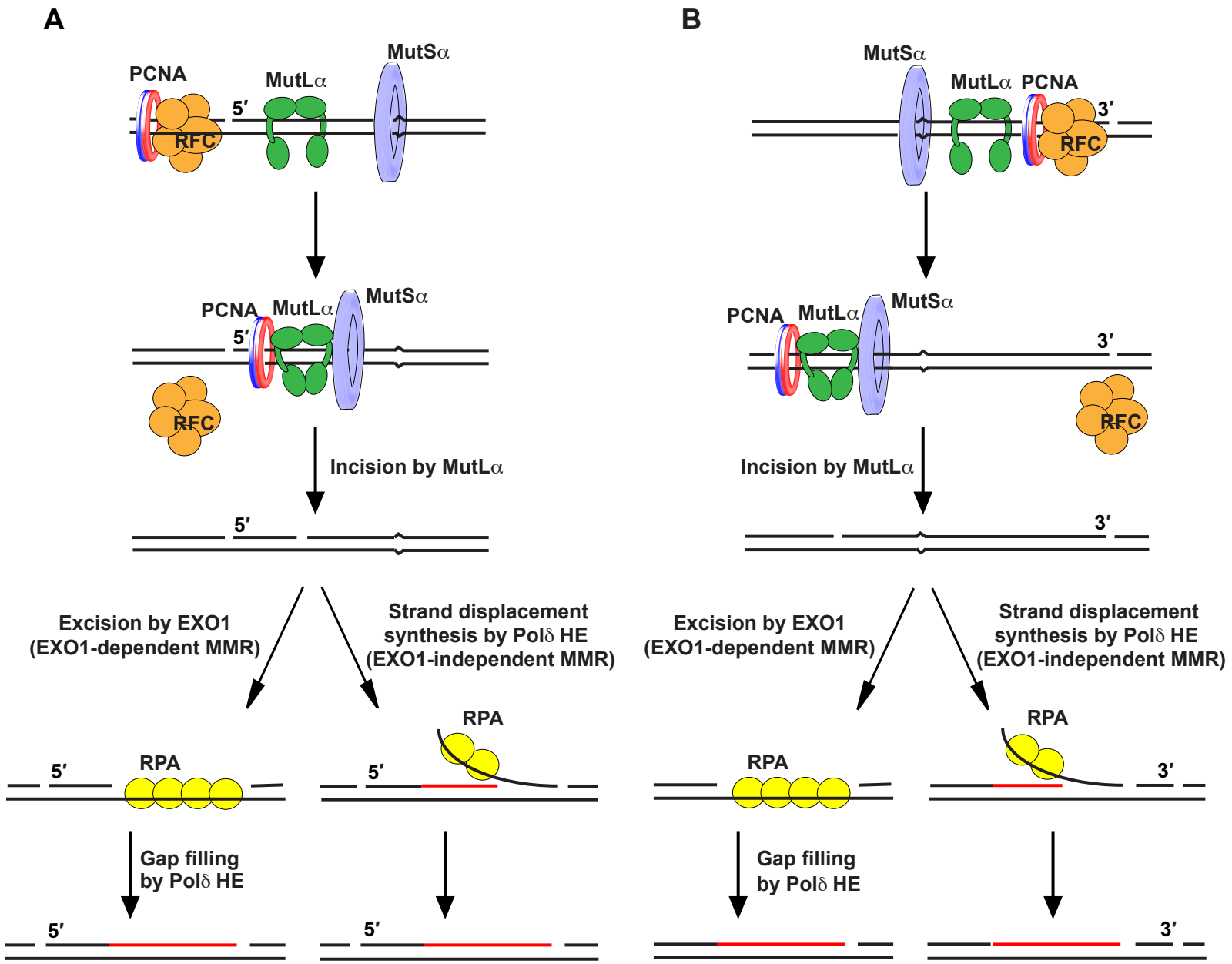
Figure legends

Figure 1. Models of EXO1-dependent and EXO1-independent MMR in human cells. The models are adapted from Kadyrov et a 2006, 2007 (39,58) and are based on the results of studies of human MMR in the reconstituted systems (39,52,53,57,58). Pol δ HE, Pol δ holoenzyme. See text (Section 3) for details.

Figure 2. Models for the activation of MutLα endonuclease in MutSα-dependent MMR. See text (Section 4) for details.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Incision by MutLα
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(EXO1-dependent MMR)
Strand displacement
synthesis by Polδ HE
(EXO1-independent MMR)
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Figure 1

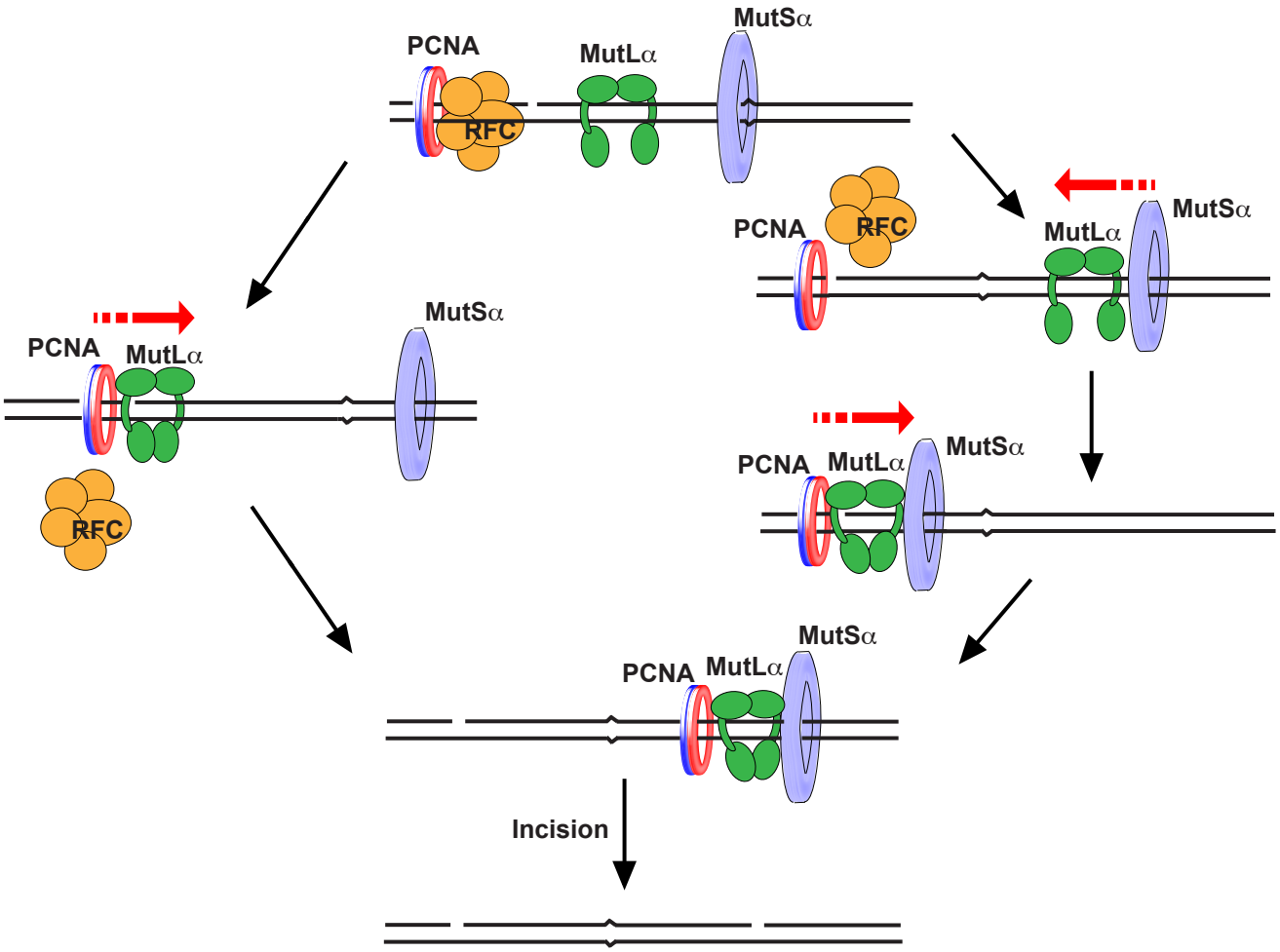
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Figure 2