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Biochemical Properties of MutL, a DNA Mismatch Repair Endonuclease

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1. Introduction

DNA mismatch repair (MMR) is one of the most widely conserved DNA repair systems, which repairs mismatched bases generated mainly by the error of DNA polymerases during replication (Friedberg, et al., 2006, Iyer, et al., 2006, Kunkel, et al., 2005, Morita, et al., 2010). MMR increases the replication fidelity by 20 to 400-fold (Schaaper, 1993). Mutations and epigenetic silencing in MMR genes cause human hereditary nonpolyposis colon cancers as well as sporadic tumors (Fishel, et al., 1995, Fishel, et al., 1994, Kane, et al., 1997, Leach, et al., 1993, Modrich, et al., 1996, Suter, et al., 2004), indicating the significance of this repair system.

To date, two types of MMR mechanisms have been clarified: one is employed by eukaryotes and most bacteria (Fig. 1A and B) (Modrich, 2006) and the other is specific to Escherichia coli and other γ-proteobacteria (Fig. 1C) (Modrich, et al., 1996). The fundamental mechanism and the required proteins in the two types of MMRs are relatively similar to each other. A mismatch is recognized by the bacterial MutS homodimer, eukaryotic MutSα (MSH2-MSH6 heterodimer), or MutSβ (MSH2-MSH3 heterodimer) (Acharya, et al., 2003, Drotschmann, et al., 2002, Gradia, et al., 1997, Gradia, et al., 1999, Lamers, et al., 2000, McCulloch, et al., 2003, Obmolova, et al., 2000, Tachiki, et al., 2000). Subsequently, the bacterial MutL homodimer or eukaryotic MutLα (MLH1-PM2 and MLH1-PM5 heterodimers in humans and yeast, respectively) is recruited to the mismatched DNA to stimulate downstream events (Acharya, et al., 2003, Kadyrov, et al., 2006). The largest difference between the two types of MMR mechanisms is in the “strand discrimination” system. Although both bases constituting the mismatch are canonical, MMR needs to identify which base is to be repaired. In eukaryotes and most bacteria, MMR directs the repair to the error-containing strand of the mismatched duplex by recognizing the strand discontinuities in the newly synthesized strand (Kadyrov, et al., 2006, Kadyrov, et al., 2007, Larrea, et al., 2010, Modrich, 2006). The termini of leading and lagging strands are thought to serve as discrimination signals. On the other hand, E. coli MMR reads the absence of methylation at the restriction site in the newly synthesized strand (Iyer, et al., 2006, Kunkel, et al., 2005, Larrea, et al., 2010). Before the site-specific DNA methylase (e.g., E. coli Dam methylase (Schlagman, et
Fig. 1. A schematic representation of MMR pathway models. (A) Eukaryotic MMR. A mismatch is recognized by MutSα, and MutLα nicks the 3'- or 5'-side of the mismatched base on the discontinuous strand. The effective incision by MutLα requires MutSα, replication factor C, proliferating cell nuclear antigen (PCNA), and ATP. The resulting DNA segment is excised by a 5'-3' exonuclease, EXO1, in cooperation with a single-stranded DNA-binding protein, replication protein A (RPA). The DNA strand is resynthesized by DNA polymerase δ and DNA ligase 1. No DNA helicase has been identified to participate in eukaryotic MMR. This mechanism is elucidated by using an in vitro reconstituted system.
The pre-existing strand discontinuity can be located on both 5'- and 3'-sides of the mismatch; therefore, there should be 5'- and 3'-nick directed MMR mechanisms. The detail has been described elsewhere (Constantin, et al., 2005, Dzantiev, et al., 2004, Fukui, 2010, Genschel, et al., 2002, Kadyrov, et al., 2009). (B) A speculative model for MMR in mutH-less bacteria. The mismatch is recognized by a MutS homodimer. After incision of the discontinuous strand by MutL, the error-containing DNA strand is removed by the cooperative functions of DNA helicases, such as UvrD, the exonucleases RecJ and ExoI, and the single-stranded DNA-binding protein (SSB). DNA polymerase III and DNA ligase fill the gap to complete the repair. Although, no studies have reported the \textit{in vitro} reconstituted system of bacterial nick-directed MMR, it has been elucidated that the endonuclease activity of MutL is required for \textit{in vivo} MMR activity (Fukui, et al., 2008). The involvement of RecJ and ExoI in this MMR system has been implicated experimentally (Shimada et al., 2010). (C) \textit{E. coli} MMR. MutS recognizes the mismatch, and MutL interacts with MutS. Subsequently, the MutH endonuclease is activated to incise the unmethylated strand at the GATC site to create an entry point for the excision reaction. DNA helicase, SSB, and several exonucleases are involved in the excision reaction. At least three models have been proposed for the mechanism by which a MutS homologue stimulates downstream events. They are the “Molecular switch”, “Stationary”, and “Translocation” models. The major difference between these models is whether a MutS homologue dissociates from the mismatch after recognizing it. Details have been provided in other publications (Kunkel, et al., 2005, Li, 2008).

al., 1986)) completes the modification of the newly synthesized strand, hemi-methylated sites exist and serve as strand-discrimination signals. In both MMR systems, a nicking endonuclease plays a central role in the strand discrimination mechanism. In eukaryotes and most bacteria, MutL homologues are thought to incise the discontinuous strand to introduce the entry or termination point of the excision reaction. In \textit{E. coli}, MutH nicks the unmethylated strand of the duplex to generate the excision entry point. After incision by MutL homologues or MutH, the error-containing strand is removed by helicases and exonucleases. DNA polymerases then resynthesize the strand, and DNA ligases seal the nick to complete the repair reaction. Although \textit{in vivo} MMR achieves mismatch- and daughter strand-specific incision, eukaryotic MutL\alpha and bacterial MutL show an apparently non-specific endonuclease activity against lesion-less DNA \textit{in vitro} (Duppatla, et al., 2009, Fukui, et al., 2008, Kadyrov, et al., 2006, Kadyrov, et al., 2007, Mauris, et al., 2009), indicating that MMR requires a sequence- or structure-non-specific endonuclease activity to introduce an excision entry point wherever it is needed. The regulatory mechanism underlying this apparently non-specific endonuclease activity has been argued (Yang, 2007). Both eukaryotic and bacterial MutL consist of N-terminal ATPase and C-terminal dimerization (also endonuclease) domains (Fig. 2) (Fukui, et al., 2008, Kadyrov, et al., 2006). The two domains are usually separated by a long, flexible linker region. This domain organization is characteristic of the GHKL ATPase/kinase superfamily that undergoes a large conformational change upon ATP binding and/or hydrolysis (Ban, et al., 1999, Dutta, et al., 2000). Generally, ATP binding and/or hydrolysis control the molecular functions of these superfamily proteins. ATP binding- and/or hydrolysis-induced conformational change is expected to be involved in the regulation of MutL endonuclease activity. Recent biochemical characterizations have demonstrated the effects of ATP binding and hydrolysis on the function of MutL (Duppatla, et al., 2009, Fukui, 2010, Genschel, et al., 2002, Kadyrov, et al., 2009).
et al., 2008, Kim, et al., 2009, Mauris, et al., 2009, Sacho, et al., 2008). In addition to ensuring mismatch-specific incision, cells also need to direct the MutL-dependent nicking reaction to the newly synthesized strand of the mismatched duplex. Interactions of MutL with other MMR proteins have been reported to participate in this regulatory mechanism. In this chapter, we review the biochemical properties of MutL endonucleases that are related to those regulatory mechanisms.

Fig. 2. A schematic representation of the domain structure of MutL homologues. ATPase, endonuclease, and dimerization domains are represented by red, blue, and yellow boxes, respectively. Numbers in parentheses indicate the length of each protein. The interdomain linker regions are shown as gray bars. The crystal structures of the human PMS2 N-terminal ATPase domain (PDB ID: 1EA6) (Guarné, et al., 2001), E. coli MutL ATPase domain (PDB ID: 1B63) (Ban, et al., 1999), Bacillus subtilis MutL C-terminal endonuclease domain (PDB ID: 3KDK) (Pillon, et al., 2010), and E. coli MutL C-terminal dimerization domain (PDB ID: 1X9Z) (Guarné, et al., 2004) are shown.

2. Structure of the C-terminal endonuclease and N-terminal ATPase domains of MutL

The C-terminal domain of MutL endonucleases contains two highly conserved sequence motifs (Fig. 3). One of them is the DQHA(x)2E(x)2E motif, which is essential for the nicking endonuclease activity (Fukui, et al., 2008, Kadyrov, et al., 2006). Aspartic acid and histidine residues in this motif are expected to coordinate one or two metal ions to catalyze the nicking reaction (Kosinski, et al., 2008, Pillon, et al., 2010, Yang, 2008). The other is the zinc-binding motif CPHGRP (Kosinski, et al., 2008), which is not essential for the nicking endonuclease activity but is required for the in vivo MMR activity (Fukui, et al., 2008, Kosinski, et al., 2008). Crystal structures of Bacillus subtilis and Neisseria gonorrhoeae MutL C-terminal domains (Namadurai, et al., 2010, Pillon, et al., 2010) revealed that their overall
Fig. 3. Amino acid sequence alignment of the C-terminal regions of MutL homologues. Red and blue boxes indicate the conserved sequence motifs, DQHA(x)E(x)E and CPHGRP, respectively. The numbers on the left and right show the distances from the N-termini for each protein. The numbers in parentheses show the number of residues that are omitted for the sake of clarity. The NCBI Entrez GI numbers of the sequences are as follows: 4505913 (Homo sapiens PMS2), 121583910 (Mus musculus PMS2), 18411951 (Arabidopsis thaliana PMS1), 46562124 (Saccharomyces cerevisiae PMS1), 17562796 (Caenorhabditis elegans PMS2), 16078768 (Bacillus subtilis MutL), 59801161 (Neisseria gonorrhoeae MutL), 15926876 (Escherichia coli MutL), 16131992 (Salmonella typhimurium MutL), and 16767605 (Staphylococcus aureus MutL).

structures, which are dimeric molecules, resemble that of the E. coli MutL C-terminal domain (Fig. 4A) (Guarné, et al., 2004, Kosinski, et al., 2005), although the E. coli MutL C-terminal domain lacks the DQHA(x)E(x)E and CPHGRP motifs. In those crystal structures, the CPHGRP motif is located adjacent to the DQHA(x)E(x)E motif to form a catalytic site in the subunit (Fig. 4B). In the crystal structure of the B. subtilis MutL C-terminal domain, two zinc ions are coordinated near the catalytic site by residues including the histidine and cysteine of the DQHA(x)E(x)E and CPHGRP motifs, respectively (Fig. 4C) (Pillon, et al., 2010). Although the function of these zinc ions has not been precisely explained, the difference between the zinc-bound and zinc-unbound forms of the B. subtilis MutL C-terminal domain demonstrated that binding of zinc ions brings about a local structural rearrangement in the catalytic site (Fig. 4C) (Pillon, et al., 2010). Since the addition of zinc ions to the reaction mixture slightly stimulates the nicking endonuclease activity of MutL (Pillon, et al., 2010), the local structural change would be a prerequisite for the formation of the active form of the catalytic site.

The N-terminal ATPase domain of MutL contains a single ATP-binding motif per subunit just like other GHKL superfamily proteins (Ban, et al., 1998, Guarné, et al., 2001). Unlike the C-terminal domain, the amino acid sequence of the N-terminal ATPase domain of the MutL endonuclease is highly homologous to that of E. coli MutL (Iino, et al., 2010). Therefore, the crystal structure of the E. coli MutL N-terminal domain can be utilized when considering the structure and function of the N-terminal domain of the MutL endonuclease. Ban and Yang described the apo and AMPPNP-bound forms of the E. coli MutL N-terminal domain (Ban, et al., 1999, Ban, et al., 1998), which clearly demonstrated the ATP binding-induced conformational change of this domain (Fig. 5A). Upon AMPPNP binding, the disordered region found in the apo structure formed ordered structures, which led to the dimerization
Fig. 4. Crystal structure of the nicking endonuclease domain of *B. subtilis* MutL (One subunit of the dimer is shown). (A) The overall structure of the endonuclease domain of *B. subtilis* MutL (blue) (zinc-bound form, PDB ID: 3KDK) (Pillon, et al., 2010) is superposed onto the dimerization domain of *E. coli* MutL (yellow) (PDB ID: 1X9Z) (Guarné, et al., 2004). The endonuclease domain is comprised of regulatory and dimerization sub-domains. The DQHA(x)E(x)4E and CPHGRP motifs are included in the dimerization sub-domain. (B) The DQHA(x)E(x)4E motif (green) is located near the CPHGRP motif (cyan). Two zinc ions (pink spheres) are coordinated by several residues including the histidine (a green stick) of the DQHA(x)E(x)4E motif and the cysteine and histidine (cyan sticks) of the CPHGRP motif. (C) The zinc ion binding induces a structural rearrangement of the catalytic site in the endonuclease domain. The zinc-bound form (colored) is superposed onto the unbound crystal forms I (white) (PDB ID: 3GAB) and II (gray) (PDB ID: 3KDG) (Pillon, et al., 2010). All structures are shown in a stereo view.

of the N-terminal domain. As with the MutL endonuclease, the crystal structure of the N-terminal domain of human PMS2 has been reported (Fig. 5B) (Guarné, et al., 2001). Intriguingly, the N-terminal domain of PMS2 bound to ATPγS even in the absence of the N-terminal domain of MLH1, which is the only report concerning ATP binding by a monomeric GHKL superfamily protein. However, it is expected that in the presence of the MLH1 subunit, ATP binding induces dimerization of the N-terminal domains. In line with this notion, a direct observation using atomic force microscopy suggested that ATP binding causes dimerization of the N-terminal domain in yeast MutLα (Sacho, et al., 2008).
3. ATP modulates the nicking endonuclease activity of MutL

The effect of ATP on the biochemical properties of the MutL endonuclease has been examined using the bacterial MutL endonuclease as a model molecule. *Thermus thermophilus* MutL stably bound one ATP molecule per subunit at a physiological concentration (2 mM) of ATP without any detectable hydrolysis activity in the absence of MutS and mismatch (Fukui, et al., 2008). Limited proteolysis indicated the ATP- or AMPPNP-dependent conformational change of *T. thermophilus* MutL (Fukui, et al., 2008).

In order to detect a nicking endonuclease activity, the covalently closed circular form of plasmid DNA is often used as a substrate (Fukui, et al., 2007). A nicking endonuclease activity converts the closed circular form into an open circular form of the plasmid DNA that can be easily separated from the closed circular form and the linearized form by agarose gel electrophoresis. Mn²⁺ facilitates the mismatch-, MutS-, clamp-, and clamp loader-independent incision of the closed circular form by non-sequence-specific MutL endonuclease activity (Duppatla, et al., 2009, Fukui, et al., 2008, Kadyrov, et al., 2006, Mauris, et al., 2009).
When *T. thermophilus* MutL was preincubated with physiological concentrations of ATP or AMPPNP before the addition of substrate DNA, the initial rate of the nicking activity was significantly reduced (Fukui, et al., 2008). This was also supported by the result of a gel electrophoretic mobility shift assay, which indicates that ATP or AMPPNP prevents the non-specific DNA binding of *T. thermophilus* MutL (Fukui, et al., 2008). The endonuclease activities of *Aquifex aeolicus* and *N. gonorrhoeae* MutL were also suppressed by the addition of ATP (Duppatala, et al., 2009, Fukui, et al., 2008). One may speculate that the observed suppressing effect is due to the chelating ability of ATP to deprive the manganese ion from MutL. However, this possibility is ruled out by the following two experimental evidences: ATP has no inhibitory effect on the endonuclease activity of the C-terminal domain of MutL (Duppatala, et al., 2009); alteration of the cysteine residue in the CPHGRP motif to an alanine results in perturbation of the suppressing effect of ATP (Fukui, et al., 2008). These results suggest that ATP-dependent suppression requires the binding of ATP to the N-terminal domain and that the zinc ion in the C-terminal domain is required for sensing ATP binding. Interestingly, AMPPNP and a mismatch facilitated the stable interaction between *T. thermophilus* MutL and MutS (Fukui, et al., 2008). The ATP-bound form of MutL would specifically interact with the MutS-DNA complex in the presence of a mismatch. Because the ATPase activity of MutL is expected to be stimulated by its interaction with MutS, the formation of the MutS-MutL complex may promote the endonuclease activity of MutL by unlocking the ATP binding-dependent suppression (Fig. 6).

![Fig. 6. A speculative model of the regulatory mechanism for the mismatch-specific enhancement of MutL nicking endonuclease activity.](https://www.intechopen.com)

Mauris and Evans reported the detailed biochemical experiment on *A. aeolicus* MutL, in which they demonstrated that ATP stimulates the nicking endonuclease activity of relatively high concentrations of *A. aeolicus* MutL in the absence of MutS and mismatch (Mauris, et al., 2009). This result suggests that the effect of ATP on the MutL endonuclease activity depends...
on the concentration of MutL, which is consistent with the previous report describing the promoting effect of ATP on the nicking endonuclease activity of eukaryotic MutLa (Kadyrov et al., 2006, Kadyrov et al., 2007). These evidences clearly indicate that ATP is utilized not only to suppress the non-specific endonuclease activity of MutL but also to actively enhance its activity. It would therefore be necessary to clarify whether ATP hydrolysis is required for enhancing the endonuclease activity. On one hand, it was reported that AMPPNP can stimulate the endonuclease activity of relatively high concentrations of A. aeolicus MutL (Mauris, et al., 2009). On the other hand, the endonuclease activity of B. subtilis MutL was not stimulated by AMPPNP even under conditions where ATP could stimulate the activity (Pillon, et al., 2010).

4. The N-terminal ATPase domain stimulates the endonuclease activity of the C-terminal domain

As described in the previous section, the endonuclease activity of MutL is modulated by ATP binding and/or hydrolysis. Because the ATP binding and endonuclease active sites are located in the N- and C-terminal domains, respectively, the interdomain interaction between them had been expected. This prediction was verified by the recent experiment using recombinant N- and C-terminal domains from A. aeolicus MutL. The N-terminal domain stimulated the endonuclease activity of the C-terminal domain by at least a 4-fold magnitude in the absence of ATP (Iino, et al., 2010). Interestingly, this promoting effect was abolished by the depletion of zinc ions from the reaction mixture or by the substitution of cysteine in the CPHGRP motif by alanine (Iino, et al., 2010). These results indicate that zinc ions are required for the N-terminal domain-dependent stimulation of the C-terminal domain. It remains to be investigated whether the zinc ions are directly involved in the interdomain interaction or whether they indirectly influence the interaction through rearrangement of the local structure.

It is expected that this interdomain interaction is involved in the ATPase cycle-dependent regulatory mechanism of MutL. Direct observation using atomic force microscopy has suggested the possible ATP binding-induced association of the N-terminal domain to the C-terminal domain (Fig. 6, middle) (Sacho, et al., 2008). Such an approach may reflect the interdomain interaction that is required for stimulating the nicking endonuclease activity. However, as mentioned above, ATP binding suppresses and ATP hydrolysis promotes the nicking endonuclease activity (Duppatla, et al., 2009, Fukui, et al., 2008, Pillon, et al., 2010). Therefore, ATP hydrolysis may create a tighter contact of the N-terminal domain with the C-terminal domain than that created by ATP binding (Fig. 6, right). Such a tight contact may stimulate the nicking endonuclease activity. Further studies are necessary to clarify whether and how ATP hydrolysis affects the structure and function of MutL endonuclease.

5. Interaction with a sliding clamp directs the MutL-dependent incision to the discontinuous strand

In the above sections, we reviewed the possible regulatory mechanism that assures the mismatch-specific nicking endonuclease activity of MutL. We also have to consider a regulatory mechanism that directs the nicking endonuclease activity of MutL to the error-containing strand of the mismatched duplex. Mismatch itself has no signal to discriminate which base is incorrect (Friedberg, et al., 2006). In vitro characterization of MMR activity in the
eukaryotic nuclear extracts has shown that discontinuities in the substrate mismatched DNA can serve as the signal to direct the MutL-dependent incision to the discontinuous strand (Kadyrov, et al., 2006, Kadyrov, et al., 2007, Modrich, 2006). In the cell, the 5’- and 3’-termini of the newly synthesized strand are expected to be used as the discrimination signal. Additionally, another question has arisen: how does MutL sense the strand discontinuity that is remote from the MutL incision site? In an in vitro reconstituted system of eukaryotic MMR, the discontinuous strand is distinguished by the cooperative function of MutLα with PCNA and replication factor C (Kadyrov, et al., 2006, Modrich, 2006). Recently, it has also been clarified that PCNA directs the incision reaction at the terminus-containing strand through direct interaction with MutLα and that replication factor C is required only for loading PCNA to the DNA (Pluciennik, et al., 2010). The MLH1 subunit of MutLα contains the PCNA-interacting motif QxxLxxFF in its C-terminal domain (Fig. 7A) (Lee, et al., 2006). The PCNA-dependent activation of MutLα was hindered by a peptide containing the PCNA-interacting motif (Pluciennik, et al., 2010). PCNA recognizes the 3’-terminus of the primed sites in DNA (Yao, et al., 2000) and tightly binds to the plasmid DNA containing a pre-existing strand break (Pluciennik, et al., 2010). PCNA has two nonequivalent faces (Gulbis, et al., 1996) and binds to the strand break with a specific orientation (Bowman, et al., 2004, Georgescu, et al., 2008). Because the interface to MutLα is on one side of the clamp (Pluciennik, et al., 2010), the interaction between PCNA and the MLH1 subunit of MutLα is expected to facilitate the asymmetric binding of the mismatched duplex with the discontinuous strand bound in the catalytic site of the PMS2 subunit (Pluciennik, et al., 2010). This may assure the daughter strand-specific incision.

Fig. 7. Amino acid sequence alignment of the PCNA- or β-clamp-interacting motifs in the C-terminal domains of MutL homologues. The consensus sequence motif is shown above the alignments. (A) The conserved residues of the PCNA-interacting motif are shown in purple. In the consensus motif, h and a represent residues with hydrophobic side-chains and aromatic side-chains, respectively. The NCBI Entrez GI numbers of the sequences are as follows: 4557757 (H. sapiens MLH1), 255958238 (M. musculus MLH1), 30680985 (A. thaliana MLH1), 6323819 (S. cerevisiae MLH1), 71991825 (C. elegans MLH1). (B) The conserved residues of the β-clamp-interacting motif in bacterial MutL are shown in orange.
Interestingly, *B. subtilis* MutL endonuclease also interacts with a β-sliding clamp (Pillon et al., 2011), a bacterial counterpart to eukaryotic PCNA, which also has two distinguishable faces (Kong et al., 1992). Most bacterial MutL endonucleases have the β-clamp-interacting motif QLxLF at the regulatory sub-domain of the C-terminal domain (Fig. 7B) (Pillon et al., 2010, Pillon et al., 2011). Mutation of this sequence motif results in defects in the *in vivo* MMR activity (Pillon et al., 2011), implying that the β-clamp-dependent activation of MutL is not necessary in the cell and that bacterial MMR also adopts a strand discrimination mechanism similar to that of eukaryotic MMR. However, MutL endonucleases from the Aquificae phylum lack the regulatory sub-domain (Iino et al., 2010). In addition, MutL endonucleases from the Thermus-Deinococcus phylum have no obvious β-clamp-interacting motifs (Fig. 7B), although they retain the regulatory sub-domain. Therefore, it should be carefully investigated whether this discrimination mechanism is universally present among all nick-directed MMRs.

6. Bacterial MutL is a homodimeric nicking endonuclease

Crystal structures of *B. subtilis* and *N. gonorrhoeae* MutL C-terminal domains, and other biochemical studies, have revealed that bacterial MutL C-terminal domains are homodimeric (Duppata et al., 2009, Iino et al., 2010, Namadurai et al., 2010, Pillon et al., 2010). Generally, linear double-stranded DNA-specific dimeric endonucleases incise both strands of the duplex. Type II (and Type IIs) restriction endonucleases and Type II DNA topoisomerases are representative of double-strand incising dimeric endonucleases. On the other hand, double-stranded DNA-specific nicking endonucleases are usually monomeric, with the exception of several structure-specific nicking endonucleases (Fukui et al., 2008, Komori et al., 2002). For example, the following linear double-stranded DNA-specific nicking endonucleases are all monomeric proteins (Table 1): N-type nicking endonucleases (e.g., N. *BspQI*), sequence-specific nicking endonucleases naturally or artificially created by mutating restriction enzymes to lose their dimerization ability (Higgins et al., 2001, Roberts et al., 2003, Xu et al., 2001, Yunusova et al., 2006, Zheleznaya et al., 2009); V-type nicking endonucleases (e.g., *E. coli* Vsr), a short patch MMR nicking endonuclease (Tsutakawa et al., 1999, Tsutakawa et al., 1999); Type I DNA topoisomerases (e.g., *E. coli* Topo I), an enzyme with a supercoil-relaxing activity (Kirkegaard et al., 1978); retrotransposon-targeting endonucleases (e.g., L1 endonuclease), a site-specific nicking endonuclease that directs the invasion of the retrotransposon (Feng et al., 1996, Feng et al., 1998, Maita et al., 2007, Weichenrieder et al., 2004); bovine DNase I, a non-specific nicking endonuclease that functions in the host defense (Suck et al., 1988); *E. coli* MutH (Ban et al., 1998), the MMR nicking endonuclease; bacterial UvrC (Nazimiec et al., 2001), a nucleotide excision repair nicking endonuclease; bacterial endonuclease V (Dalhus et al., 2009), a deaminated DNA-specific nicking endonuclease; and bacterial and eukaryotic AP endonucleases (Hosfield et al., 1999, Mol et al., 2000), an abasic site-specific nicking endonuclease. Known DNA repair systems other than MMR all adopt a monomeric nicking endonuclease to introduce the entry point for the excision reaction. Therefore, the dimerization ability of the MutL C-terminal domain might be related to the strand-discrimination mechanism of bacterial MMR.
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Table 1. Linear double-stranded DNA-specific nicking endonucleases. 1Structural analyses have revealed that AP endonucleases, retrotransposon-targeting endonucleases, and DNase I are closely related to each other. 2Serratia nuclease can convert the covalently closed circular form of plasmid DNA not only to the linear form but also to the open circular form; however, the major product of this nuclease is the double-strand break. Serratia nuclease also incises single-stranded DNA, and dimerization is not essential for the nuclease activity. 3MutL shows no structural similarity to other known endonucleases. 4These nicking endonucleases introduce the starting point for the excision reaction in damaged or error-containing single-stranded DNA. Among these DNA repair nicking endonucleases, only MutL forms a dimer.
The homodimeric structure of bacterial MutL prompts the question of how the symmetric homodimer generates asymmetric nicking products. As with eukaryotic MutLa, the asymmetry would be derived from the nature of the heterodimer. Eukaryotic MutLa has a single catalytic site for the endonuclease activity. On the other hand, bacterial MutL contains two catalytic sites that are apparently equivalent to each other. It may be possible that bacterial MutL dissociates from the substrate DNA before the catalysis of the second strand incision because of its low velocity, or that the binding of the product to the one subunit induces a non-productive binding mode of the substrate to the other subunit. Alternatively, as proposed by Namadurai et al., the inverted arrangement of the bacterial MutL C-terminal domain dimer may enable interactions with other MMR proteins to interfere with one of the two active sites during the reaction (Namadurai, et al., 2010).

7. Conclusion

In this chapter, the biochemical properties of MutL endonucleases are reviewed, with an emphasis on their regulatory mechanisms. The regulatory mechanism needs to ensure both mismatch- and daughter-strand-specific incisions. The ATPase cycle-dependent conformational and functional changes of the MutL endonucleases are expected to play a central role in these mechanisms. Since the ATPase cycle-dependent conformational change would involve the rearrangement of the interaction between the N- and C-terminal domains, the structural analysis of full-length MutL is urgently required. For the structural analysis, MutL homologues from some thermophilic bacterium may be suitable because of the lack of flexible interdomain linker region as well as their extreme thermostability. However, the interdomain linker region plays a significant role in the in vitro function of eukaryotic MutLa (Gorman, et al., 2010). Therefore, it is necessary to carefully judge whether the obtained information is universal among all MutL endonucleases.

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9. References


Ban, C. & Yang, W., Crystal structure and ATPase activity of MutL: implications for DNA repair and mutagenesis, Cell, Vol. 95, No. 4, (November 1998), pp. 541-552, ISSN 0092-8674

Ban, C. & Yang, W., Structural basis for MutH activation in E. coli mismatch repair and relationship of MutH to restriction endonucleases, EMBO Journal, Vol. 17, No. 5, (March 1998), pp. 1526-1534, ISSN 0261-4189


Feng, Q., Schumann, G. & Boeke, J. D., Retrotransposon R1Bm endonuclease cleaves the target sequence, *Proceedings of the National Academy of Sciences of the United States of America*, Vol. 95, No. 5, (March 1998), pp. 2083-2088, ISSN 0027-8424


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Pillon, M. C., Miller, J. H. & Guarné, A., The endonuclease domain of MutL interacts with the β sliding clamp, *DNA Repair (Amst)*, Vol. 10, No. 1, (January 2011), pp. 87-93, ISSN 1568-7856


Yang, W., Human MutLα: the jack of all trades in MMR is also an endonuclease, *DNA Repair (Amst)*, Vol. 6, No. 1, (January 2007), pp. 135-139, ISSN 1568-7864


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