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Review Article

DNA Mismatch Repair in Eukaryotes and Bacteria

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DNA mismatch repair (MMR) corrects mismatched base pairs mainly caused by DNA replication errors. The fundamental mechanisms and proteins involved in the early reactions of MMR are highly conserved in almost all organisms ranging from bacteria to human. The significance of this repair system is also indicated by the fact that defects in MMR cause human hereditary nonpolyposis colon cancers as well as sporadic tumors. To date, 2 types of MMRs are known: the human type and *Escherichia coli* type. The basic features of the former system are expected to be universal among the vast majority of organisms including most bacteria. Here, I review the molecular mechanisms of eukaryotic and bacterial MMR, emphasizing on the similarities between them.

1. Introduction

DNA mismatch repair (MMR) is a highly conserved DNA repair system (Table 1) that greatly contributes to maintain genome stability through the correction of mismatched base pairs. The major source of mismatched base pairs is replication error, although it can arise also from other biological processes [1]. In *Escherichia coli*, MMR increases the accuracy of DNA replication by 20–400-fold [2]. Mutations and epigenetic silencing in MMR genes have been implicated in up to 90% of human hereditary nonpolyposis colon cancers [3–8], indicating the significance of this repair system. Postreplicative MMR is performed by the long-patch MMR mechanism in which multiple proteins are involved and a relatively long tract of the oligonucleotide is excised during the repair reaction [9, 10]. In contrast, particular kinds of mismatched base pairs are repaired through very short-patch MMR in which a short oligonucleotide tract is excised to remove the lesion [11–13]. In this paper, I refer to long-patch MMR as MMR.

Currently, 2 types of MMR mechanisms have been elucidated: one is expected to be employed by eukaryotes and the majority of bacteria, and the other is specific to *E. coli* and closely related bacteria. As shown in Figures 1(a) and 1(b), MMR in eukaryotes and most bacteria directs the repair to the error-containing strand of the mismatched duplex by recognizing the strand discontinuities. On the other hand, as shown in Figure 1(c), *E. coli* MMR reads the absence of methylation as a strand discrimination signal. In both MMR systems, strand discrimination is conducted by nicking endonucleases. MutL homologues from eukaryotes and most bacteria incise the discontinuous strand to introduce the entry or termination point for the excision reaction. In *E. coli*, MutH nicks the unmethylated strand of the duplex to generate the entry point of excision.

Here, I first review the overviews of MMR systems in *E. coli*, eukaryotes, and the majority of bacteria. Second, I refer to the molecular features of MutS and MutL homologues, the key enzymes in MMR. Third, molecular mechanisms for strand-discrimination and excision reaction are discussed. Finally, I introduce the cellular functions of MMR other than postreplication mismatch correction.

2. Overview of Methyl-Directed MMR in *E. coli*

The *E. coli* MMR system has been well characterized and reconstituted using recombinant proteins (Figure 1(c)) [14]. In this system, a mismatched base is recognized by a MutS homodimer. A MutL homodimer interacts with the MutS-DNA complex, and then a MutH restriction endonuclease is activated by MutL. The MMR system needs to discriminate the newly-synthesized/error-containing strand; however, a mismatched base itself contains no such signal. The *E. coli*

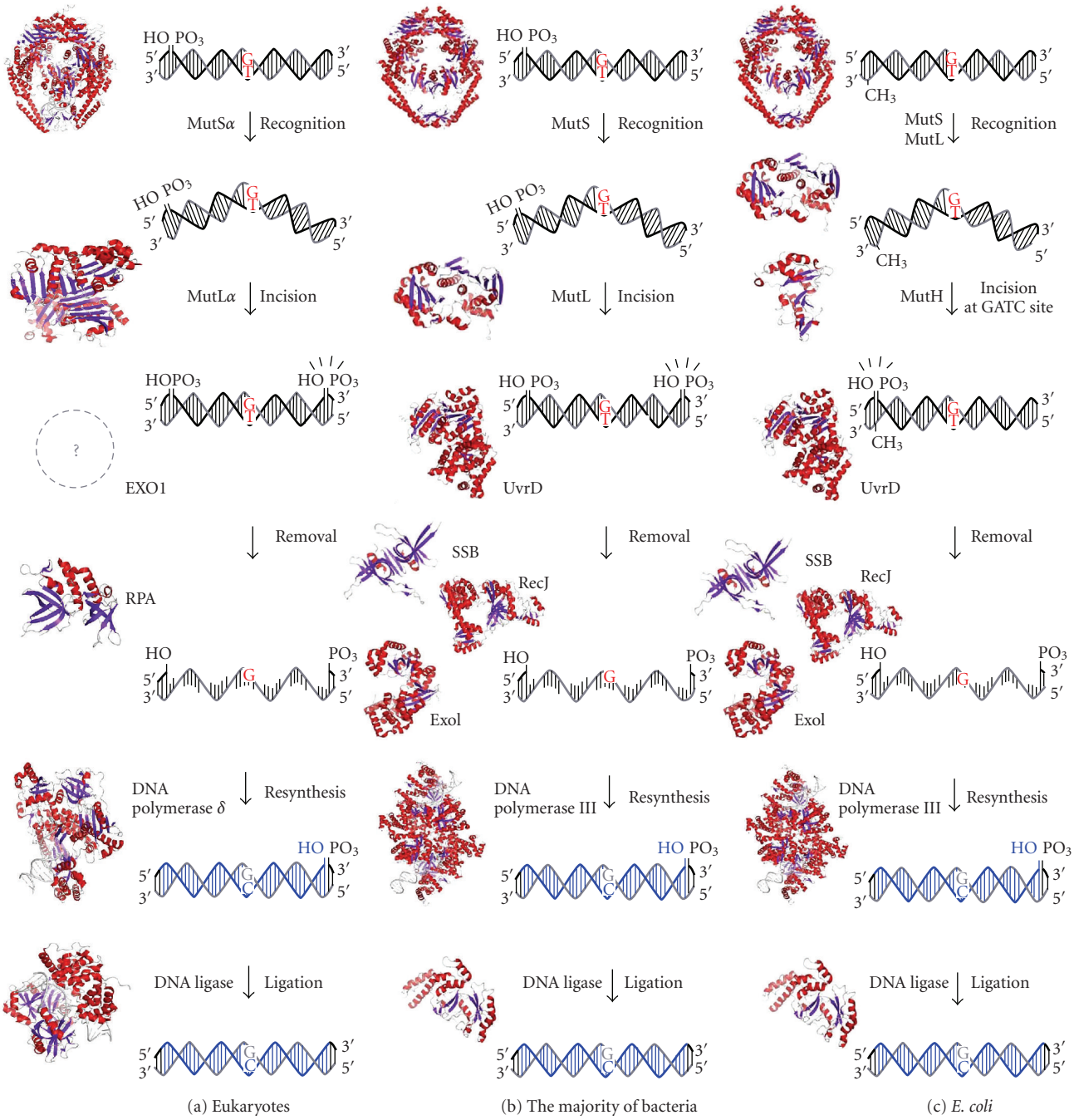

Figure 1: A schematic representation of MMR pathway models. (a) Eukaryotic MMR. A DNA mismatch is generated by the misincorporation of a base during DNA replication. MutSα recognizes base-base mismatches and MutLα nicks the 3′- or 5′-side of the mismatched base on the discontinuous strand. The resulting DNA segment is excised by the EXO1 exonuclease, in cooperation with the single-stranded DNA-binding protein RPA. The DNA strand is resynthesized by DNA polymerase δ and DNA ligase 1. (b) MMR in *mutH*-less bacteria. Mismatched bases are recognized by MutS. After the incision of 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. (c) *E. coli* MMR. MutS recognizes mismatched bases, and MutL interacts with and stabilizes the complex. Then, MutH endonuclease is activated to incise the unmethylated GATC site to create an entry point for the excision reaction. DNA helicase, a single-stranded DNA-binding protein, and several exonucleases are involved in the excision reaction. PDB IDs of crystal structures in this figure are 2O8B (human MutSα), 1H7S (human MutLα), 1L1O (human RPA), 3IAY (human DNA polymerase δ), 1X9N (human DNA ligase 1), 1E3M (bacterial MutS), 1B63 (bacterial MutL), 2AZO (*E. coli* MutH), 2ISI (bacterial UvrD), 2ZXO (bacterial RecJ), 3C95 (bacterial ExoI), 2CWA (bacterial SSB), 2HQA (bacterial DNA polymerase III), and 2OWO (bacterial DNA ligase).

Table 1: Distribution of MMR proteins.

Molecular function	*Thermus thermophilus*	*Escherichia coli*	*Saccharomyces cerevisiae*	*Homo sapiens*
Mismatch recognition	MutS	MutS	MutSα (MSH2/MSH6) MutSβ (MSH2/MSH3)	MutSα (MSH2/MSH6) MutSβ (MSH2/MSH3)
Strand incision	β-clamp[*1] clamp-loader[*1]	—	PCNA RFC	PCNA RFC
	MutL		MutLα (MLH1/PMS1) MutLγ[*2] (MLH1/MLH3)	MutLα (MLH1/PMS2) MutLγ[*2] (MLH1/MLH3)
Strand incision	—	MutH	—	—
Match making	MutL	MutL	MutLα (MLH1/PMS1) MutLβ (MLH1/MLH2) MutLγ (MLH1/MLH3)	MutLα (MLH1/PMS2) MutLβ (MLH1/PMS1) MutLγ (MLH1/MLH3)
Strand excision (single-stranded DNA-binding)	SSB	SSB	RPA	RPA
Strand excision (exonuclease)	RecJ ExoI	RecJ ExoI ExoVII ExoX	EXO1[*3]	EXO1[*3]
Strand excision (helicase)	UvrD	UvrD	—	—
Repair synthesis	DNA polymerase III	DNA polymerase III	DNA polymerase δ	DNA polymerase δ

[*1]The involvement of bacterial clamp and clamp-loader in the strand incision reaction has not yet been confirmed. [*2]It is demonstrated that the endonuclease motif in MLH3 is responsible for *in vivo* MMR [83]; however, the endonuclease activity of MutLγ has not yet been confirmed biochemically. [*3]In yeast and human, EXO1 has the 5′-flap endonuclease activity in addition to 5′–3′ exonuclease activity.

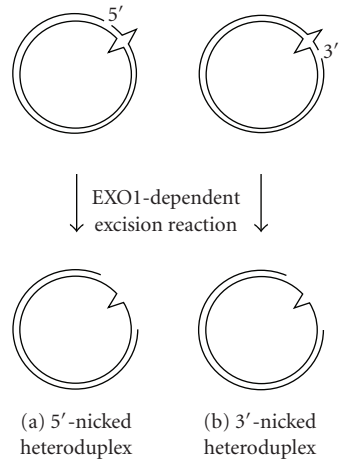

Figure 2: Bidirectionality of eukaryotic MMR. The 5′-nicked (a) and 3′-nicked (b) heteroduplexes are used as model substrate. The shorter path is chosen to remove the mismatched base. The 5′–3′ exonuclease activity of EXO1 is required for excision reaction in both 5′- and 3′-nicked heteroduplexes.

MMR system utilizes the absence of methylation at the restriction site to direct the repair to the newly synthesized strand. MutH nicks the unmethylated strand at the hemimethylated GATC site to introduce an entry point for the excision reaction [15–17]. The error-containing strand is removed by helicases [18] and exonucleases [19–21], and a new strand is synthesized by DNA polymerase III and ligase. The absence of methylation serves as a signal for the discrimination of the error-containing strand, and hence, *E. coli* MMR is called methyl-directed MMR. Although homologues of *E. coli* MutS and MutL exist in almost all organisms, no homologue of *E. coli* MutH has been identified in the majority of organisms including eukaryotes and most bacteria (Table 1) [22].

3. Overview of Eukaryotic MMR

In eukaryotes, strand discontinuity serves as a signal that directs MMR to the discontinuous strand of a mismatched duplex (Figure 1(a)). In newly synthesized strands, discontinuities can exist as 3′-ends or termini of Okazaki fragments. These termini of the DNA strand may function as strand discrimination signals *in vivo*. For the biochemical characterization of eukaryotic MMR, nicked plasmid DNAs have been used as a model substrate containing a strand discontinuity, on which several reviews elaborated [10, 23, 24]. For this assay system, the shorter path from a nick to the mismatch is removed by the excision reaction, indicating that 5′- and 3′-directed MMRs are distinct (Figure 2) [25–27]. Intriguingly, the 5′–3′ exonuclease activity of exonuclease 1 (EXO1) is required for both 5′- and 3′-directed strand removals [28, 29]. The reason for the apparently contradictory requirement of 5′–3′ exonuclease activity for the 3′-discontinuity-directed excision reaction had been unknown because it was thought that strand discontinuity was also the entry point for excision reaction. This issue was resolved by the discovery that the human MutL homologue MutLα (MLH1-PMS2 heterodimer) and yeast MutLα (MLH1-PMS1 heterodimer)

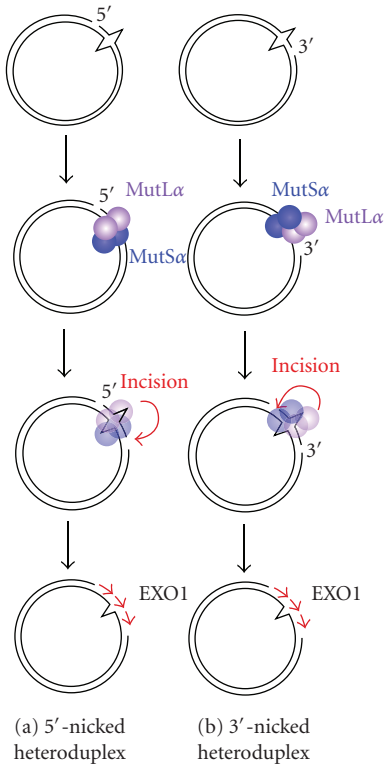

Figure 3: The 5′-nick-(a) and 3′-nick-(b) directed eukaryotic MMR. After recognition of a mismatched base by MutSα, MutLα incises the discontinuous strand of the heteroduplex in a mismatch-MutSα-, PCNA-, RFC-, and ATP-dependent manner [10, 30, 31]. Incisions by MutLα occur dominantly on the distal side of the mismatched base relative to the pre-existed strand break although it can also occur proximal to the mismatch [30].

harbor latent endonuclease activity that nicks the discontinuous strand of the mismatched duplex in a MutSα-, PCNA-, RFC-, and ATP-dependent manner (Figure 3) [10, 30, 31]. In eukaryotic 5′- and 3′-directed MMR, the 3′- and 5′-sides of a mismatch are incised, respectively, by MutLα, and the 5′–3′ exonuclease activity of EXO1 removes the DNA segment spanning the mismatch, that is, in eukaryotic 3′-directed MMR, the preexisting strand discontinuity does not serve as an entry point for the excision reaction, and the entry point is introduced by the endonuclease activity of MutLα (Figure 3(b)).

Intriguingly, MutLα is essential for 3′-nick-directed MMR but it is dispensable for 5′-nick-directed MMR, although MutLα possesses an enhancing effect on 5′-nick-directed MMR [32, 33]. This result indicates that there is an alternative pathway for 5′-nick-directed MMR. *In vitro* experiments suggest that MutLα-independent 5′-nick-directed human MMR requires at least 3 proteins, MutSα, EXO1, and replication protein A (RPA) [32].

4. Overview of MMR in *mutH*-Less Bacteria

The DQHA(X)$_2$E(X)$_4$ motif in the C-terminal domain of the PMS2 subunit of human MutLα comprises a metal-binding site that is essential for the endonuclease activity of MutLα and MMR activity of nuclear extract [30]. Except for *E. coli* and closely related bacteria, most *mutH*-lacking bacteria possess MutL homologues that contain this metal-binding motif [30, 34]. Therefore, the molecular mechanism established on the basis of the results obtained from eukaryotic MMR systems is expected to be universal for organisms lacking *mutH*. In agreement with this prediction, several studies demonstrated that MutL homologues from *mutH*-less bacteria, for example, *Thermus thermophilus*, *Aquifex aeolicus*, and *Neisseria gonorrhoeae*, possess endonuclease activity that is dependent on the DQHA(X)$_2$E(X)$_4$ motif [34–36]. Furthermore, in *T. thermophilus*, it is clarified that the endonuclease activity of MutL is essential for *in vivo* DNA repair activity [34]. Thus, the molecular mechanism of MMR in *mutH*-less bacteria is expected to resemble that of eukaryotic one (Figure 1(b)).

5. Molecular Functions of MutS Homologues

The amino acid sequence of MutS is highly conserved among bacteria regardless of the presence and absence of *mutH* [37–39]. Bacterial MutS forms a homodimer and recognizes mispaired bases and short insertion/deletion loops [9, 40, 41]. Eukaryotes possess several mismatch-recognizing MutS homologues: MSH2, MSH3, and MSH6. These 3 homologues contain amino acid sequences homologous to bacterial MutS and form 2 heterodimers, namely, MutSα (MSH2/MSH6) and MutSβ (MSH2/MSH3). MutSα recognizes single base-base mismatches and short insertion/deletion loops while MutSβ is responsible for the repair of relatively large insertion/deletion loops that contain ~16-mer excess nucleotides [42].

The crystal structures of C-terminal dimerization domain-deleted bacterial MutS [37, 38] and human MutSα [43] were solved in complex with mismatched DNA (Figure 4). The structures revealed that the mismatch-recognition mechanisms of bacterial MutS and eukaryotic MutSα fundamentally resemble each other. The bacterial MutS homodimer/DNA complex shows a functionally asymmetric protein dimer in which only 1 of the 2 subunits recognizes the mismatched or unpaired base. Thus, although bacterial MutS binds to double-stranded DNA as a homodimer, its functionality is heterodimeric.

Bacterial MutS and human MutSα recognize the heteroduplex by stacking the mismatched base with a phenylalanine residue (Phe36 and Phe432 in *E. coli* MutS and human MSH6, resp.) that is intercalated from the minor groove side [38] (Figure 4). This phenylalanine residue is perfectly conserved among bacterial MutS homologues and eukaryotic MSH6 [37], and the alteration of this residue to an alanine results in a drastic decrease in the mismatch-recognition ability of *Thermus aquaticus* MutS [49]. The adjacent glutamate residue (Glu38 and Glu434 in *E. coli* MutS and human MSH6, resp.) also plays a central role in mismatch recognition by forming a hydrogen bond with the mismatched base (Figures 5(a)–3(c)) [38]. This glutamate

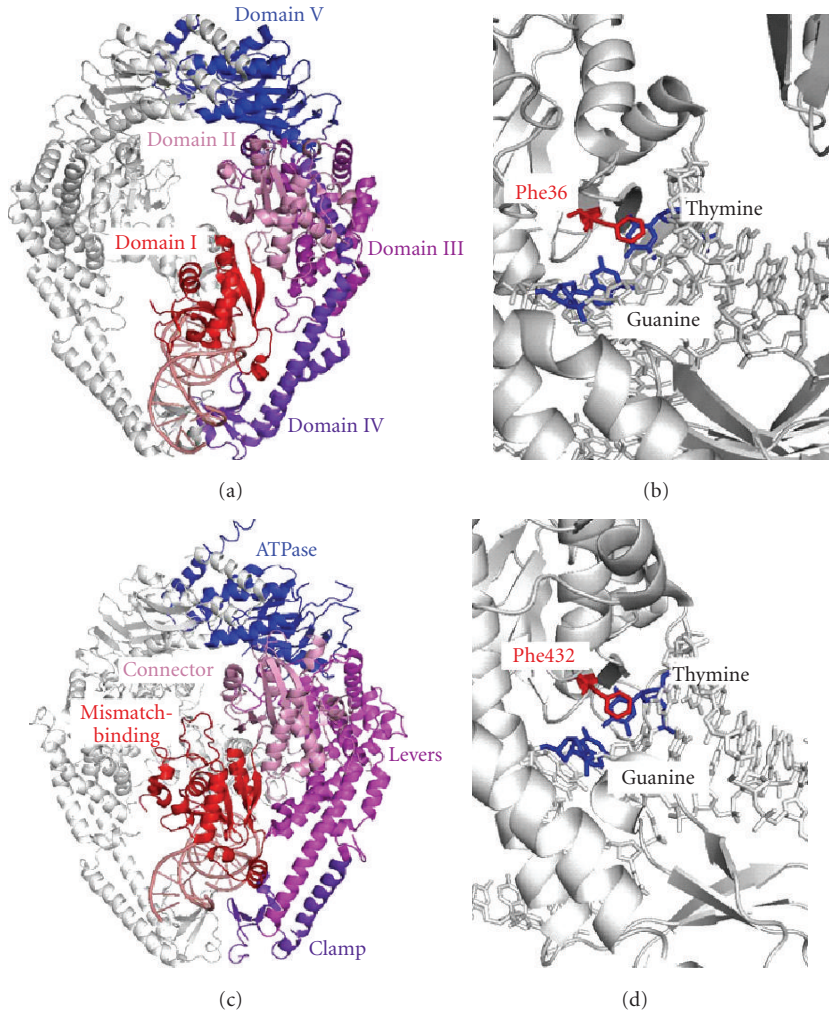

Figure 4: Crystal structures of MutS-mismatch complex. (a) Crystal structure of *E. coli* MutS bound to a G:T-mismatched heteroduplex (PDB ID: 1E3M). One of the 2 subunits of *E. coli* MutS is shown in color. DNA is shown in salmon. Domains I, II, III, IV, and V are shown in red, pink, violet, purple blue, and blue, respectively. Domains I, IV, and V are responsible for mismatch recognition, double-stranded DNA binding, and dimerization/ATP binding, respectively. (b) The mismatch-recognition site in *E. coli* MutS-G:T mismatch complex. The mismatch-recognizing phenylalanine residue (Phe36) and G:T mismatch are shown in red and blue, respectively. (c) Crystal structure of human MutSα (PDB ID: 2O8B), which is comprised of full-length MSH2 and a protease-resistant fragment of MSH6 lacking the first 340 amino acid residues. MSH2 is shown in white and MSH6 is in color. Mismatch-binding, Connector, Levers, Clamp, and ATPase domains are colored in red, pink, magenta, purple, and blue, respectively. (d) The mismatch-recognition site in human MutSα-G:T mismatch complex. Phe432 and G:T mismatch is shown in red and blue, respectively.

residue is also conserved among bacterial MutS and eukaryotic MSH6 [37], and the mutation of this glutamate into an alanine or a glutamine abolishes *in vivo* MMR activity [50–52]. Elimination of this hydrogen bond by replacing the thymidine with 2,4-difluorotoluene, which lacks the N3, resulted in the decrease in mismatch selectivities of *E. coli* MutS and yeast MutSα [50, 52].

The excellent crystallographic analyses of *E. coli* MutS complexes with various kinds of mismatched heteroduplexes remarkably enhanced our understanding of the mismatch recognition mechanism at an atomic resolution [53]. MutS recognizes a wide range of mismatches in a common manner. Heteroduplexes bound by MutS homologues are sharply kinked with the minor groove wide-opened (Figure 5(d)), which allows the mismatched base to be in close contact with the phenylalanine and the glutamate residues of the mismatch-recognition domain. The carbonyl oxygen (OE2) of Glu38 in *E. coli* MutS forms a hydrogen bond with N3 of mismatched pyrimidine or N7 of mismatched purine base [53]. It is expected that N7 of mismatched purine or OE2 of the glutamate is protonated. As previously discussed, this might be the reason why the mutation of Glu38 to Gln in *E. coli* MutS eliminates MMR activity though Gln also can form hydrogen bonds [53]. The acidity of the glutamate but not glutamine might be suitable for the required pK_a shift. Interestingly, E38A mutant of *E. coli* MutS exhibited the enhanced DNA-binding activity to a perfectly matched homoduplex DNA [50]. Schofield et al. proposed the idea

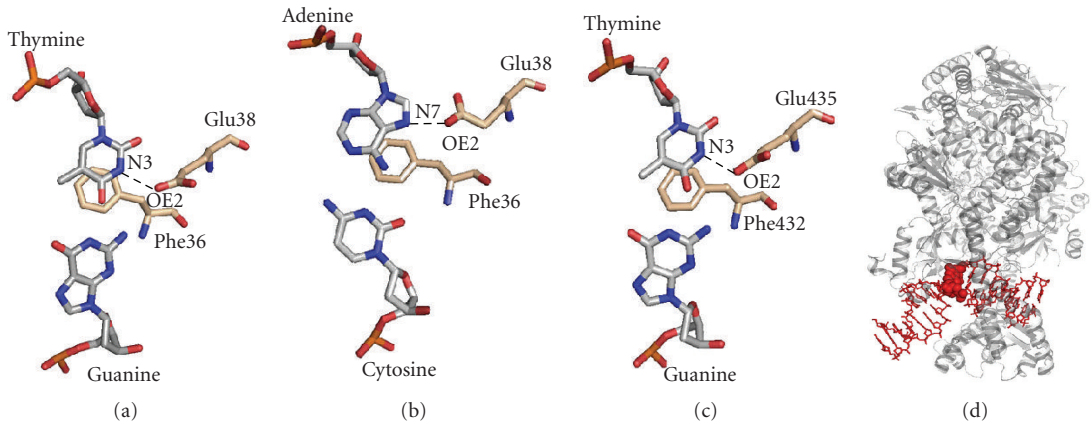

Figure 5: Mismatch recognition mode of MutS. (a) G:T mismatch bound to *E. coli* MutS (PDB ID: 1E3M). (b) C:A mismatch bound to *E. coli* MutS (PDB ID: 1OH5). Cytosine residue is in a syn conformation. (c) G:T mismatch bound to human MutSα (PDB ID: 2O8B). (d) Side view of the *E. coli* MutS-mismatch complex (PDB ID: 1E3M). The mismatched duplex is sharply kinked in the complex with MutS. MutS and mismatched DNA are colored grey and red, respectively. The mismatched G and T are shown in the sphere model.

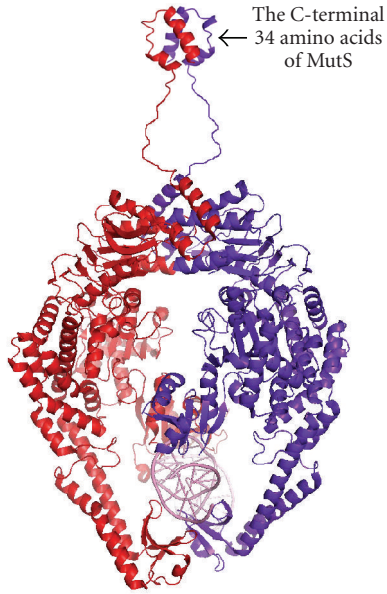

Figure 6: The model for full length of *E. coli* MutS dimer. The crystal structure of the C-terminal 34 amino acids of *E. coli* MutS (2OK2) [44] was connected to the C-termini of *E. coli* MutS (residues 2-800) structure (1E3M).

that Glu38 would take part in the promotion of DNA kinking by destabilizing the unkinked DNA-MutS complex through the electrostatic repulsion of the Glu side-chain with phosphate backbone [50].

The lack of a crystal structure for MutS homologues with a homoduplex has prevented us from further understanding the mechanism; however, atomic force microscopy is a powerful tool that can be used to investigate the events that occur during the initial substrate binding by MutS homologues. Observations of the *E. coli* MutS-DNA complex using atomic force microscopy revealed that the DNA is bent at the perfectly matched site while it is bent and unbent at the mismatched site [54]. Taken together with previous biochemical and crystallographic data, it is proposed that MutS nonspecifically binds to DNA and bends it in order to search for a mismatch, when it encounters a mismatch it kinks the DNA at the mismatched site and finally forms an ultimate recognition complex in which the DNA is unbent [54]. In order to explain why an unbent conformation is more stable at a mismatch than at a homoduplex site, Wang et al. speculated the possibility that a mismatched base is flipped out upon mismatch recognition by MutS [54]. Base flipping is one of the major base-recognition mechanisms observed among base-processing enzymes such as DNA glycosylases or methyltransferases [55]. Although further experimental evidence should be required, this idea may be attractive especially when we remember the recent report that a nonenzymatic protein, alkylguanine alkyltransferase-like protein, also flips out the methylated base upon substrate recognition [56, 57].

The mismatch-recognizing property of MutS homologues is closely related to their ATP-binding/hydrolyzing activity [58–63]. MutS homologues contain a Walker's ATP-binding motif in each subunit that is formed through the association of the subunits. It has been shown that *E. coli* MutS exchanges ADP to ATP upon mismatch binding then undergoes a conformational change to form a sliding clamp [61, 63]. The ATP-binding-dependent formation of the sliding clamp was also confirmed in eukaryotic MutS homologues [63–66]. Several studies revealed that the ATPase activity of the *E. coli* MutS homodimer is also heterodimeric [67, 68]. One of the 2 nucleotide-binding sites exhibits a high affinity to ADP, and the other shows a high affinity to ATP [60, 69, 70], therefore, we should discriminate between the different adenine nucleotide-binding forms of MutS homologues in MMR. Recently, a computational study using normal-mode analysis was applied to assess the conformational dynamics of *E. coli* MutS and human MutSα [71]. Normal-mode analysis is one of the simulation

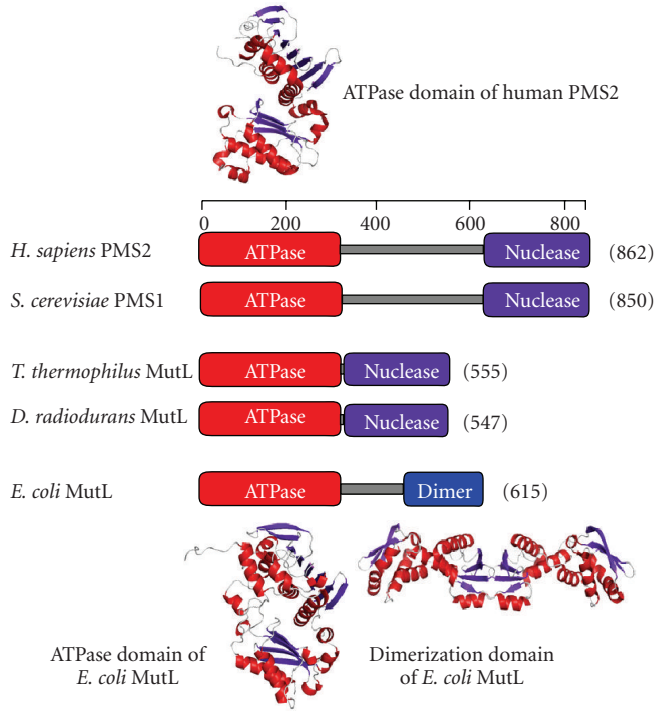

Figure 7: (a) A schematic representation of the domain structure of MutL homologues. ATPase, nuclease, and dimer indicate the ATPase, endonuclease, and dimerization domains, respectively. The crystal structures of N-terminal ATPase domain of human PMS2 (PDB ID: 1EA6) [45], ATPase domain of *E. coli* MutL (PDB ID: 1B63) [46], and C-terminal dimerization domain of *E. coli* MutL (PDB ID: 1X9Z) [47, 48] are shown.

methodologies which can deal with the dynamics of large molecules [72]. The analyses suggested the existence of the distinct conformational states which are expected to reflect the functional cycles including DNA scanning, mismatch recognition, repair initiation, and sliding along DNA after mismatch recognition [71]. These computational studies can provide start points for further experiments.

It is thought that the sliding clamp of MutS leaves the mismatch and diffuses along the DNA to activate the downstream reactions, which is called the "moving" or "*cis*" model [23, 74]. The "moving" model is supported by the result that blockages between the mismatch and the nick prevent *in vitro E. coli* MMR [75]. In contrast, another mechanism, which is called the "stationary" or "trans" model, is also proposed to account for the activation of downstream reactions [23, 74]. In the "stationary" model, MutS remains bound at the mismatch after recognition, and the ATPase activity of MutS is required for verification of mismatch recognition. The "stationary" model is supported by the observation that MutS-mismatch complex can activate MutH on separate homoduplex molecule [67].

The dynamism and transiency of the ternary complex of MutS (MutSα)-MutL (MutLα)-mismatch had prevented us from characterizing their physical interactions. Hydrogen/deuterium exchange mass spectrometry (DXMS) is suitable to study the interactions in large and transient complexes. DXMS analysis of the formation of *E. coli* MutS-MutL-mismatch complex revealed that a relatively small region in domain II (Figure 4) of MutS serves as an interface for binding MutL [76]. On the basis of the structural analyses of the MutS and MutL N-terminal domains, Wei Yang and coworkers also predicted this region to be a MutL-interacting site [37]. Although the sequence similarity of this region is limited, a structurally conserved region in the MSH2 subunit of *Saccharomyces cerevisiae* MutSα is also essential for interaction with MutLα [76].

E. coli MutS proteins exist not only in a dimeric form but also in a tetrameric form at high concentrations [41, 77]. The C-terminal domain (the last 53 amino acids in *E. coli* MutS) is responsible for the tetramerization of full-length MutS. Although the crystallographic analyses were achieved by using C-terminal truncations, it has been pointed out that the deletion of the C-terminal domain causes defects in mismatch recognition, MutH stimulation, and *in vivo* MMR activity [77–79]. On the basis of the crystallographic analysis of the C-terminal domain (Figure 6), tetramer-disrupting mutants of *E. coli* MutS were prepared [44]. Examination of the effects of those mutations revealed that dimerization but not tetramerization of the MutS C-terminal domain is essential for *in vivo* mismatch repair [44].

6. Molecular Functions of MutL Homologues

Bacterial MutL homologues exist as homodimers [47] while eukaryotic MutL homologues form the heterodimers MutLα, MutLβ, and MutLγ [80–83]. It is suggested that a large portion of eukaryotic MMR is performed by MutLα. In contrast,

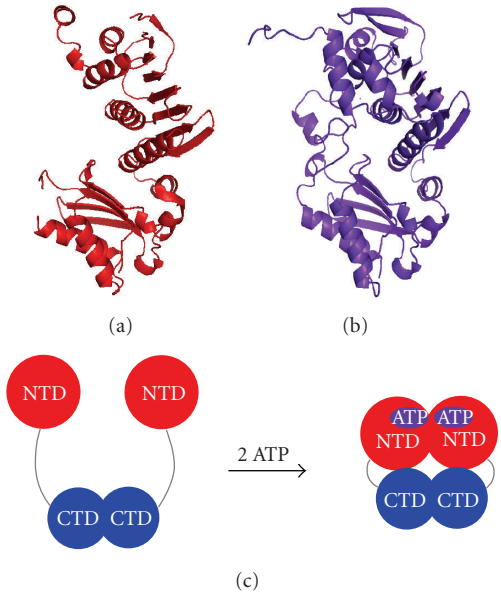

Figure 8: Crystal structures of the apo form ((a) PDB ID: 1BKN) and ADPNP-bound form ((b) PDB ID: 1B63) of the *E. coli* MutL N-terminal domain. (c) A schematic representation of a model for the ATP-dependent conformational change of full-length MutLα [73]. NTD and CTD indicate the N-terminal ATPase domain and the C-terminal endonuclease domain, respectively. In the apo form of MutLα, the PMS2 and MLH1 subunits dimerize via their C-terminal domains. ATP binding induces the dimerization of the N-terminal domain and condensation of the molecule.

there is only a slight effect of inactivation of MutLβ or MutLγ on *in vivo* MMR activity. Crystallographic and biochemical analyses revealed that the bacterial MutL homodimer is comprised of an N-terminal ATPase/DNA-binding domain and a C-terminal dimerization/DNA-binding domain (Figure 7) [47, 84]. Eukaryotic MutL homologues are expected to have the same domain organization as bacterial MutL, except for the prolonged interdomain linker (Figure 7) [45, 85].

The N-terminal ATPase domain contains a single ATP-binding motif per subunit. MutL homologues belong to the GHKL ATPase superfamily that includes homologues of DNA gyrase, Hsp90, and histidine kinase in addition to MutL homologues [46, 86]. The GHKL superfamily proteins undergo large conformational changes upon ATP binding and/or hydrolysis. X-ray crystallographic analysis clarified the ATP-binding-dependent conformational change of the N-terminal domain of *E. coli* MutL (Figure 8(a)) [46]. In addition, study using atomic force microscopy revealed that full-length *S. cerevisiae* MutLα can exist in several ATP-binding species with specific conformations in a solution [73]. The conformational change of the full-length MutL homologue seems to involve the interaction between the N-terminal and C-terminal domains (Figure 8(c)). ATP-dependent conformational changes are also implicated for bacterial MutL endonucleases [34, 87]. Such ATPase-cycle-dependent conformational changes in MutL homologue should be necessary to perform the MMR reaction [88–90].

The C-terminal domain of MutL homologues is the endonuclease domain in *mutH*-less organisms [30, 31]. The inactivation of the metal-binding motif in the C-terminal domain of *Homo sapiens* PMS2, *S. cerevisiae* PMS1, *T. thermophilus* MutL, *A. aeolicus* MutL, and *N. gonorrhoeae* MutL diminishes their endonuclease activity [34–36]. Eukaryotic MutLα and bacterial MutL show apparently nonspecific endonuclease activity against lesionless DNA, indicating that MMR requires the sequence- or structure-nonspecific endonuclease activity to introduce excision entry point wherever it is needed [30, 34]. The regulatory mechanism of this apparently nonspecific endonuclease activity has been argued [10, 91].

The endonuclease activity of MutL homologues is affected by the binding of ATP to their N-terminal domain. Isolated *T. thermophilus* MutL tightly binds ATP in the absence of the MutS-mismatch complex and the ATP-binding form of MutL has decreased endonuclease activity against perfectly matched substrates *in vitro* [34]. The *in vitro* endonuclease activities of *A. aeolicus* and *N. gonorrhoeae* MutL are also suppressed by the addition of ATP [34, 35]. In addition, *T. thermophilus* MutL is stably associated with a MutS-mismatch complex in the presence of ATP [34]. Since it has been known that the ATPase activity of MutL is activated by its interaction with MutS, formation of MutS-mismatch-MutL complex is expected to stimulate the endonuclease activity of MutL by canceling the ATP-dependent suppression (Figure 9). In other words, ATP may be utilized to suppress the apparently nonspecific endonuclease activity of MutL until it is required. However, it remains to be determined whether the regulatory mechanism is used by other MutL homologues, including eukaryotic MutLα, because the endonuclease activity of MutLα is enhanced by the addition of ATP instead of being suppressed [30, 31]. Interestingly, it is clarified that ATP stimulates the endonuclease activity of a relatively high concentration of *A. aeolicus* MutL in the absence of a MutS-mismatch complex [36], suggesting that the effect of ATP on the MutL endonuclease activity depends on the concentration of MutL. This finding also strongly indicates that the ATPase activity of MutL is required for its endonuclease activity, that is, ATP is utilized not only to suppress the nonspecific endonuclease activity of MutL but also to actively enhance its activity.

The C-terminal endonuclease domain of MutL homologues contains the highly conserved motif, CPHGRP. Mutations in this motif result in the deficiency of *in vivo* and *in vitro* MMR activities [34, 85]. Bioinformatic analysis indicated that this motif takes part in the formation of the metal-binding motif and it resembles a metal-dependent transcriptional regulator [85]. Using biochemical procedures, the C-terminal domain of the human PMS2 subunit of MutLα was demonstrated to bind a zinc ion [85]. Although the detailed functions of this zinc ion remain unknown, the involvement of the CPHGRP motif in the ATP-dependent conformational change in *T. thermophilus* MutL is suggested [34].

As mentioned above, recent biochemical studies on the endonuclease activity of MutL homologues have been achieved by using homologues from thermophilic bacteria, such as *T. thermophilus*, *A. aeolicus*, and *Thermotoga*

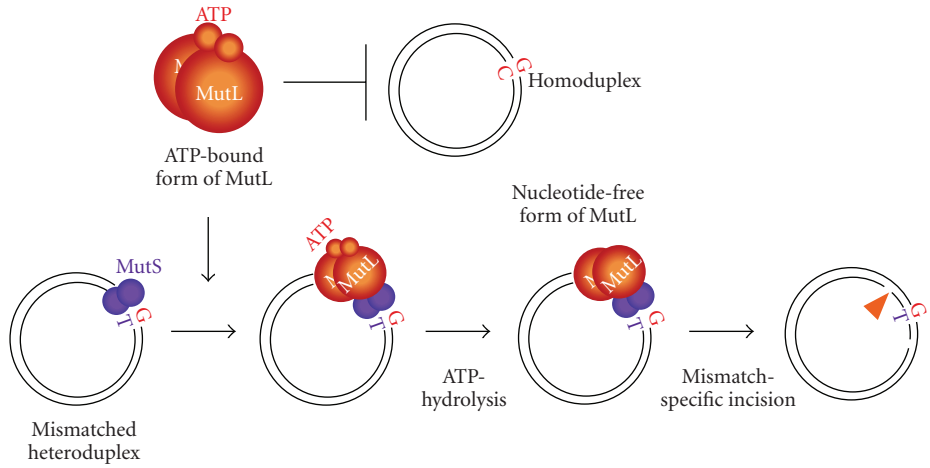

Figure 9: A model for the ATPase-cycle-dependent regulation of bacterial MutL endonuclease activity. Free MutL exists as an ATP-bound form whose endonuclease activity is inactive, but preferably binds to a MutS-mismatch complex. The interaction with the MutS-mismatch complex induces the ATP hydrolysis of MutL, resulting in the stimulation of its endonuclease activity. Adapted from the work of Fukui et al. [34].

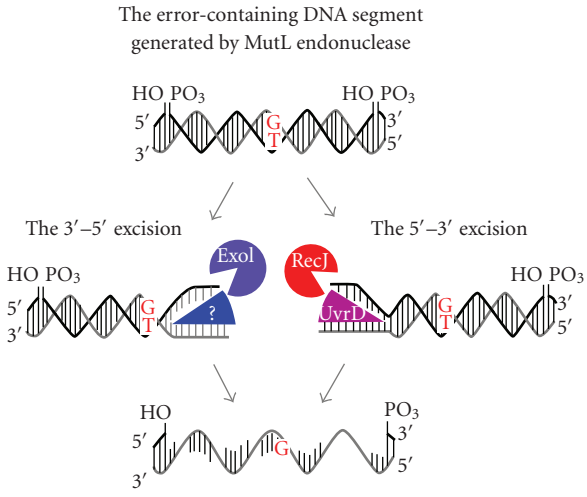

Figure 10: Two parallel pathways of excision reaction in *T. thermophilus*. RecJ (red) and ExoI (purple) are thought to be responsible for the 5′- and 3′-directed excision, respectively. UvrD helicase (magenta) functions in cooperation with RecJ. DNA helicase (blue) which translocates 5′ to 3′ direction has been unknown.

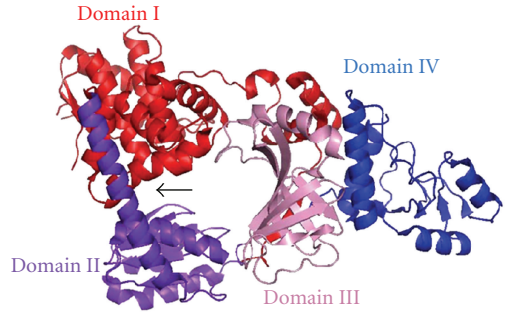

Figure 11: Crystal structure of *T. thermophilus* RecJ (PDB ID: 2ZXR). Full-length *T. thermophilus* RecJ is comprised of domains I–IV and forms a ring-like structure. The catalytic active site is located in the cavity between domains I and II as indicated by an arrow. Domain III shows a structural similarity to the oligonucleotide/oligosaccharide-binding fold that is often found in single-stranded DNA-binding proteins. The ring-like structure and oligonucleotide/oligosaccharide-binding fold will ensure the high processivity and strict specificity for single-stranded DNA.

maritima [34, 36, 87, 92]. Proteins from these bacteria are extremely stable and suitable for physicochemical examinations including crystallographic analysis. In addition, a variety of gene manipulating procedures are established in *T. thermophilus*. *T. thermophilus* may be one of the ideal model organisms for the study of nick-directed MMR.

7. Strand Discrimination in Nick-Directed MMR

Accumulating evidence indicates that a pre-existing strand break can serve as a signal to discriminate the error-containing strand in eukaryotic MMR [27, 30]. Since newly synthesized strands always contain strand break as 3′-ends or 5′-termini of Okazaki fragments, these ends can be utilized as strand discrimination signals *in vivo*. This is consistent with the observation that MutSα-dependent yeast MMR corrects mismatches more efficiently in the lagging strand than in the leading strand [93]. As mentioned above, MutLα is responsible for the strand-discrimination by nicking the discontinuous strand of the mismatched duplex (Figures 1 and 3). Interestingly, it is demonstrated that MutLα incises the discontinuous strand at a distal site from the pre-existing strand break. How does MutLα discriminate the discontinuous strand at a site distant from the strand-discrimination signal? One possible explanation is that MutSα (or MutSβ) and MutLα are loaded onto

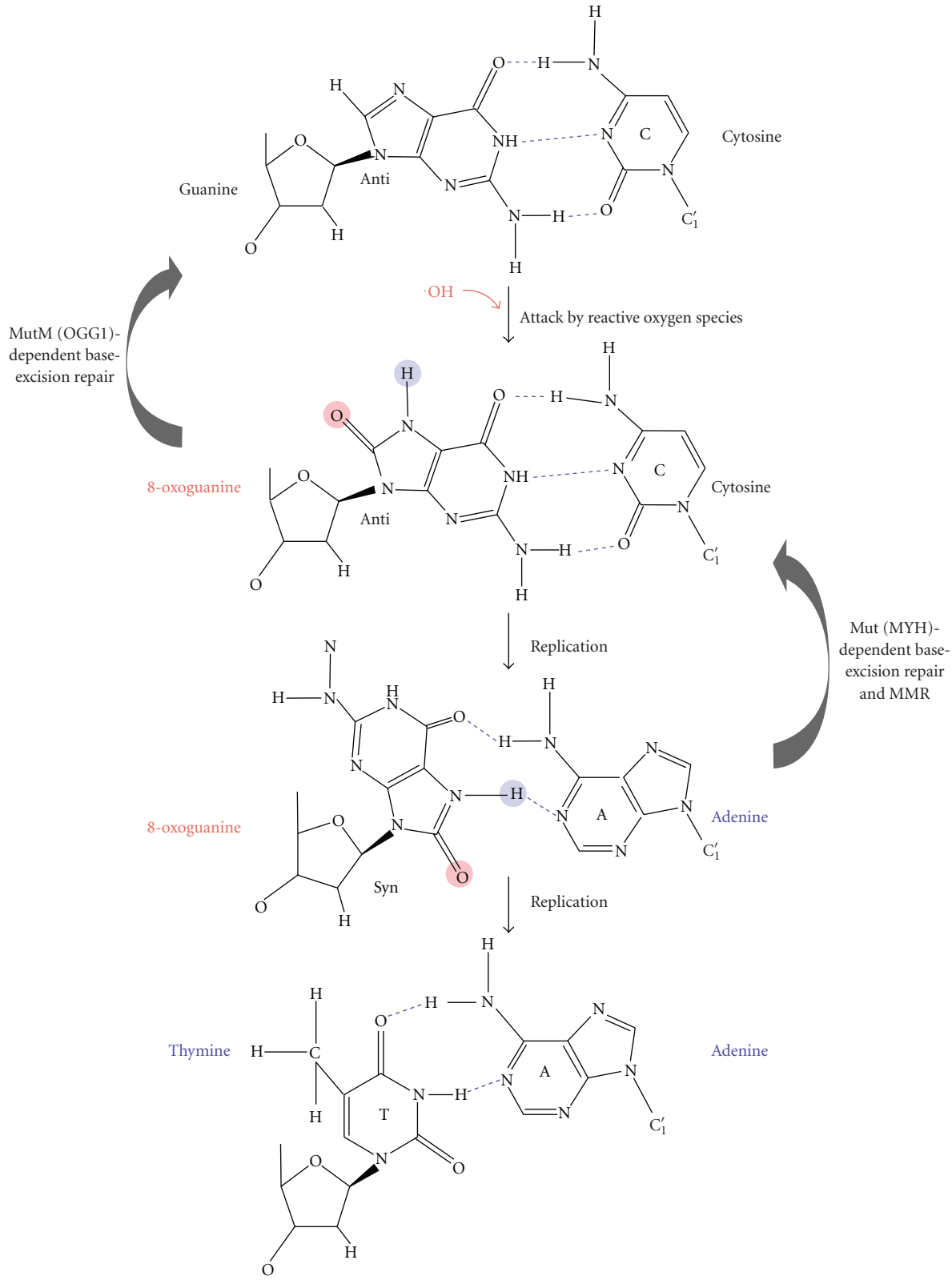

Figure 12: Prevention of 8OG-induced G:C-T:A transversion mutations. The 8OG base is one of the major forms of oxidative DNA damage and can be generated by reactive oxygen species. Since 8OG can pair not only with C but also with A, it causes a G:C-T:A transversion through DNA replication. MutM (OGG1)- and MutY (MYH)-dependent base-excision repair pathways are known to remove the 8OG and A from 8OG:C and 8OG:A pairs, respectively. MMR is also responsible for the removal of A from 8OG.

the substrate DNA through their interactions with the replication machinery such as proliferating cell nuclear antigen (PCNA), replication factor C (RFC), and/or DNA polymerase δ to render the newly synthesized strand in the catalytic site of the MutLα endonuclease domain [23, 94, 95]. It is known that MSH3 and MSH6 contain the PCNA-interacting motif $QX_2(L/I)X_2FF$ [96]. In fact, several studies have demonstrated the associations of MutSα and MutSβ with PCNA [95, 97, 98]. In addition, PCNA and RFC are necessary not only for repair synthesis [99] but also for the mismatch-provoked incision and excision reactions [30, 100]. Inhibitors of PCNA abolish 3′-nick-directed excision and 40–50% of 5′-nick-directed excision [99–101]. The excision reaction of MutLα-independent 5′-nick-directed MMR may be performed independently of PCNA. In *mutH*-less bacteria, the C-terminal region of MutS contains the putative β-clamp-binding motif QLSFF [102]. The deletion of this region abolishes the *in vitro* interaction of MutS with the β-clamp and *in vivo* MMR activity [103]; furthermore, this interaction is necessary for the *in vivo* localization of MutS and MutL in response to mismatches [103]. These interactions may also be responsible for the strand discrimination in bacterial nick-directed MMR.

8. Downstream Events in Nick-Directed MMR

The excision reaction of *in vitro* eukaryotic MMR is performed by the 5′–3′ single-stranded DNA-specific exonuclease EXO1. To date, EXO1 is a unique exonuclease that is involved in eukaryotic MMR [30, 104]. In addition, no reports have identified the MMR-related eukaryotic DNA helicase. The exonuclease activity of EXO1 is enhanced by its direct interaction with MutSα [99]. As mentioned above, MutSα forms a sliding clamp and diffuses along the DNA after mismatch recognition. The purpose of the diffusion of MutSα from the mismatch may be to activate EXO1 at the 5′-terminus. In *mutH*-less bacteria, *A. aeolicus* MutL enhances the DNA helicase activity of UvrD [105] whose amino acid sequence is ubiquitous among bacteria. Furthermore, a genetic study implied the simultaneous involvements of the 5′–3′ exonuclease RecJ and a 3′–5′ exonuclease ExoI in *T. thermophilus* MMR [106]. In *mutH*-less bacteria, the error-containing DNA segment generated by the endonuclease activity of MutL may be removed bi-directionally by the cooperative function of multiple exonucleases and helicases (Figure 10).

The termination of the EXO1-dependent excision reaction in eukaryotic 3′-nick-directed and MutLα-dependent 5′-nick-directed MMR can be directed by 3′-termini that are pre-existing and newly introduced by MutLα, respectively (Figure 3). In contrast, excision termination in MutLα-independent 5′-nick-directed MMR appears to employ a relatively complicated mechanism, because the termination of the excision reaction is not directed by the terminus of the DNA strand. Excision termination in MutLα-independent 5′-nick-directed MMR is conducted by the inhibitory function of RPA, a single-stranded DNA-binding protein [32]. In *mutH*-less bacteria, the mechanism for terminating the excision reaction remains unknown. The implication of the involvements of 5′–3′ and 3′–5′ exonucleases [106] raises the possibility that the excision termination of 5′- and 3′-nick-directed MMR in *mutH*-less bacteria is directed by the 3′- and 5′-termini of the DNA strand that are introduced by MutL (Figure 10). Biochemical and structural studies on the exonucleases are required to further understand the excision reaction. Structural analyses of the RecJ 5′–3′ exonuclease from *T. thermophilus* were successfully performed [20, 107]; thus, proteins from *T. thermophilus* are known to be suitable for physicochemical examinations [108, 109]. The structure of RecJ consists of 4 domains that form a ring-like structure with the catalytic site in the center of the ring (Figure 11). Based on this structure, the molecular basis for the high processivity and substrate specificity of this enzyme was discussed [107]. In addition, a detailed biochemical study on the *T. thermophilus* ExoI was also performed [106]. It is expected that, unlike other model organisms, *T. thermophilus* possesses only a single set of 5′–3′ and 3′–5′ exonucleases [106]. Hence, *in vitro* and *in vivo* experiments concerning the excision reaction would be relatively straightforward in this bacterium. It will be intriguing to examine whether MutS can stimulate the exonuclease activities of RecJ and ExoI.

9. Other Functions

Mismatch base pairs can arise not only from replication error but also from other biological processes including homeologous recombination, oxidation, and methylation of bases. Long-patch MMR also has a role in the repair machinery for those mismatches.

The involvement of MMR proteins in the suppression of homeologous recombination, that is, the strand exchange between nonidentical DNA molecules, has been reported [110, 111]. Inhibition of homeologous recombination contributes to genome integrity by limiting the invasion of foreign replicons and the excessive intracellular rearrangement of genome. Although the requirement of MutS and MutL homologues for this inhibitory function has been demonstrated, the downstream reactions following the recognition of the mismatch had not been described. Recently, it is suggested that the endonuclease activity of MutLα is required for this system [112].

Oxidative damage is one of the major spontaneously arising forms of DNA damage. Aerobic cells yield reactive oxygen species via respiration events that attack biomolecules such as proteins, lipids, and DNAs [113]. The attack of reactive oxygen species on DNA bases generates oxidized bases including 8-oxoguanine (8OG) (Figure 12) [114]. An 8OG base can pair not only with cytosine but also with adenine [115]. An 8OG:A pair can be converted to a T:A pair through replication, forming a G:C-T:A transversion mutation. To prevent this mutational process, base-excision repair employs MutM (OGG1 in eukaryotes) and MutY (MYH in eukaryotes) glycosylases that excise 8OG and adenine from 8OG:C and 8OG:A pairs, respectively [115]. *In vitro* and *in vivo* studies indicated that bacterial and eukaryotic MMR can recognize an 8OG:A pair as a substrate

and remove the adenine residue because the mismatched adenine residue exists in the newly synthesized strand [116, 117], that is, MMR can perform the same role as the MutY (MYH)-dependent base-excision repair pathway (Figure 12).

O^6-methylguanine (O^6MeG) is generated by the action of S_N1-alkylating agents, such as *N*-methyl-*N*-nitrosourea and *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine that are used in cancer chemotherapy [118]. O^6MeG can pair with thymine, resulting in a G:C-A:T transition mutation through replication. Although the major repair activity for O^6MeG is derived from O^6MeG methyltransferase and/or its homologues [56, 119], MutSα can also bind to an O^6MeG:T mismatch [43, 120]. However, if the O^6MeG is in the template strand of the duplex, MMR does not remove the lesion. The accumulation of the complex of unrepaired O^6MeG with MMR proteins is thought to result in the induction of apoptosis [121] through the crosstalk between MMR proteins and check point kinases [120].

10. Conclusions

In this paper, the molecular mechanism of widely conserved human-type MMR has been described. Since the accumulating evidence indicates the similarities of basic features among bacterial and eukaryotic MMR, *mutH*-less bacteria may serve as a model organism for biochemical and structural studies of MMR proteins.

The structural analyses on initial recognition complex should be required for further understanding of mismatch-recognition mechanism of MutS homologues. It would be beneficial to identify the amino acid residue which is responsible for the kinking of heteroduplex by MutS homologues. Such mutant might be utilized for the crystallographic analysis of the initial recognition complex. In order to understand the ATP-dependent functional cycles of MutL endonucleases, structural analyses on the full-length MutL homolgoues should be necessary. Since MutL endonucleases from several thermophilic bacteria have relatively short linker region between N-terminal and C-terminal domains [34, 36, 92, 105], these proteins are expected to be suitable for crystallographic analyses. However, we should not underestimate the importance of this interdomain linker region. This region might be responsible for the protein-protein interaction or domain-domain interaction just like intrinsically disordered proteins [122]. It also remains to be clarified how MutL endonucleases discriminate the discontinuous strand of mismatched heteroduplex. Further experiments using *in vitro* reconstituted system may provide the key findings to understand the mechanism. Discrimination between the "moving" and "stationary" models should also be argued in this context.

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