Chapter 18

DNA Mismatch Repair in Mammals

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Chapter Outline

1. INTRODUCTION AND BRIEF HISTORY

Preserving genomic integrity is essential for all organisms to survive and reproduce. Ensuring high-fidelity replication is critical for maintaining genome stability as all organisms are frequently exposed to exogenous and endogenous sources of DNA damage. In eukaryotes, the replicative DNA polymerases Polδ and Polε select the correct nucleotide for incorporation with high precision; however, once every 10^4 to 10^5 nucleotides, an error is made [1]. These base–base mispairs, for example, T opposite G (T:G), are corrected by the exonuclease proofreading function of replicative polymerases when the abnormal geometry of a mismatched base pair slows the extension of DNA synthesis triggering the editing function. More problematic are insertion/deletion loops (IDLs) or indels. If left unrepaired, IDLs give rise to insertions or deletions and accompanying frameshift mutations. These arise in regions of nucleotide repeats, for example, a run of A's. During replication, the template strand can slip out of register with respect to the newly synthesized strand. These DNA loops generally escape the proofreading function of Polδ and Polε as they can be located away from the polymerase active site. DNA mismatch repair (MMR) targets both base–base mismatches that have escaped proofreading, and IDLs restoring the original parental sequence in an excision pathway referred to as post-replication repair. As such, MMR contributes between 100- and 1000-fold to the overall fidelity of replication. Given its central role in assuring replication fidelity, it is not surprising that MMR is extensively conserved. Unexpectedly, it is absent in *Actinobacteria*, many *Archaea*, *Helicobacter* and *Campylobacter* and most *Mollicutes* including *Mycobacterium tuberculosis* [2].

Loss of MMR confers a mutator phenotype in which the rate of spontaneous mutation is increased 50–1000-fold. In addition, the accumulation of IDLs leads to microsatellite instability (MSI) characterized by genomic expansion or contraction in regions containing 1–4 nucleotide repeat sequences. The central role of MMR in mutation avoidance and genome stability is underscored by the fact that inactivating mutations in several key MMR genes, most commonly *MSH2* and *MLH1*, but also *PMS2* and *MSH6*, are linked to Lynch syndrome, also known as hereditary nonpolyposis colorectal cancer [3,4]. Lynch syndrome, one of the most common hereditary cancers in humans, is inherited in an autosomal dominant fashion in which carriers are heterozygous for the germline-inactivating mutation. Loss of the functioning allele by epigenetic silencing or mutation results in colorectal carcinoma, endometrial carcinoma, and other cancers. Furthermore, epigenetic silencing of MMR genes, most commonly *MLH1*, is associated with a subset of sporadic tumors [5]. Lynch syndrome tumor cells frequently exhibit MSI due to the loss of MMR-induced IDL correction, as is the case in bacteria and fungi [6].

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This review focuses on MMR in mammalian cells—the molecular mechanisms that operate in post-replication repair, its regulation, and the role of MMR proteins in DNA-damage signaling. MMR proteins have important functions in a number of other cellular processes not discussed here, for example, (1) repairing DNA double-strand breaks (DSBs) and regulating homologous recombination in both meiosis and mitosis [7]; (2) promoting triplet repeat expansion in neurodegenerative diseases [8]; and (3) promoting somatic hypermutation in the variable regions of immunoglobulin genes in activated B cells [9,10]. The MMR literature is vast, and the reader is directed to several reviews on MMR [1,11,12]. Much of what is known about MMR has been learned from genetic, biochemical, and structural studies of MMR in unicellular organisms including its earliest description in studies of repair and recombination in bacteria and meiotic recombination in fungi. Mouse models have provided important information (reviewed in Ref. [13]), and studies in *Saccharomyces cerevisiae* inform virtually all aspects of MMR, although only a small subset are cited here. Reviews of bacteria and yeast MMR highlight ongoing advances in these experimentally tractable systems [1,12,14–17].

2. POST-REPLICATION MISMATCH REPAIR

2.1 Overview

MMR directed against replication errors has three main steps: (1) mismatch recognition in newly synthesized DNA; (2) DNA excision targeted to the newly synthesized DNA strand in the vicinity of the mismatch; and (3) high-fidelity, error-free DNA synthesis to fill the single-strand gap, thereby restoring an intact duplex with no errors (see Fig. 18.1). Excision exclusively on the newly synthesized strand is critical for mutation avoidance as indiscriminate excision on the template strand is mutagenic. The reconstitution of MMR in vitro with purified human proteins has facilitated dissection of the molecular pathway [18–20].

In discussing the key features of mammalian MMR, it is helpful to draw comparisons with MMR in *Escherichia coli* and fungi, particularly *S. cerevisiae*, that encompass many of the essential features of the mammalian pathway. Two key MMR proteins in *E. coli*, MutS and MutL, are homodimers encoded by single genes, *mutS* and *mutL*, respectively (Table 18.1). In eukaryotes multiple genes have arisen through gene duplication yielding MutS and MutL homologs that are heterodimeric proteins. This combinatorial aspect of eukaryotic MMR proteins facilitates their multifunctional roles mentioned earlier. MMR is initiated when *E. coli* MutS or eukaryotic MutS homologs (MSH) MutSα (MSH2–MSH6) and MutSβ (MSH2–MSH3) recognize and bind to mismatched DNA. *E. coli* MutS repairs seven of eight base–base mispairs and small IDLs; C:C mispairs are recalcitrant to MMR and are substrates for base excision repair (BER). MutSα and MutSβ have overlapping but distinct substrate specificities. MutSα targets all base–base mispairs except C:C as well as +1 IDLs and, to a lesser extent, +2 IDLs, whereas MutSβ targets 1–4 nt IDLs and a limited subset of base–base mispairs including C:C, A:A, and possibly G:G mispairs (see [21] and references cited therein). In addition to a mismatch-binding domain (MBD), MutS proteins have two nucleotide-binding domains (NBDs). ATP binding and hydrolysis license subsequent steps of MMR involving MutS and MutL proteins by modulating the binding interaction of MutS on DNA and its interaction with MutL in prokaryotes and, most frequently, MutLα (MLH1–PMS2) in human cells or Mlh1–Pms1 in *S. cerevisiae*. The designation PMS (post-meitoic segregation) derives from the earliest identification of MutL homologs in studies of meiotic recombination in fungi [16]. A second MutL homolog, MutLγ (MLH1–MLH3) is important for meiotic recombination, but also has a minor role in MMR based on genetic and biochemical data from *S. cerevisiae*, mice, and human cells (reviewed in Ref. [12]).

Activation of MutS and MutL proteins on mismatched DNA leads to nuclease excision exclusively on the newly synthesized strand. This is well understood for *E. coli* and a few closely related gamma-proteobacteria but not for most other organisms. Targeted excision of the newly synthesized strand in *E. coli* is mediated by the MutH protein, an endonuclease activated by MutL [15]. MutH nicks the unmethylated strand at hemimethylated GATC sequences that are substrates for the Dam methyltransferase and are present in newly replicated DNA that has not yet been methylated post replication. Thus, MutH nicking is restricted to the newly synthesized, transiently unmethylated strand. A 3′–5′-helicase, UvrD, also activated by MutL unwinds from the nick providing a single-strand substrate for multiple single-strand exonucleases possessing 5′–3′- or 3′–5′-directionality. In this way, MMR is bidirectional and can respond to strand discontinuities in the chromosome that are located on either side of the mismatch (reviewed in Ref. [22]). In most organisms including eukaryotes, no MutH homolog exists, and in eukaryotes, the only exonuclease known to function in MMR is EXO1, an obligate 5′–3′-exonuclease that can function on dsDNA. The C-terminus of scExo1 contains an Msh2-binding domain [23] and an Mlh1-interacting protein (MIP) box [24]. Thus, MMR can utilize EXO1 for 5′-nick-directed MMR, but must utilize a novel excision mechanism to insure strand specificity of repair and bidirectionality. The unexpected discovery of a latent endonuclease activity in the human MutL homolog PMS2 that is activated for cleavage via interactions with

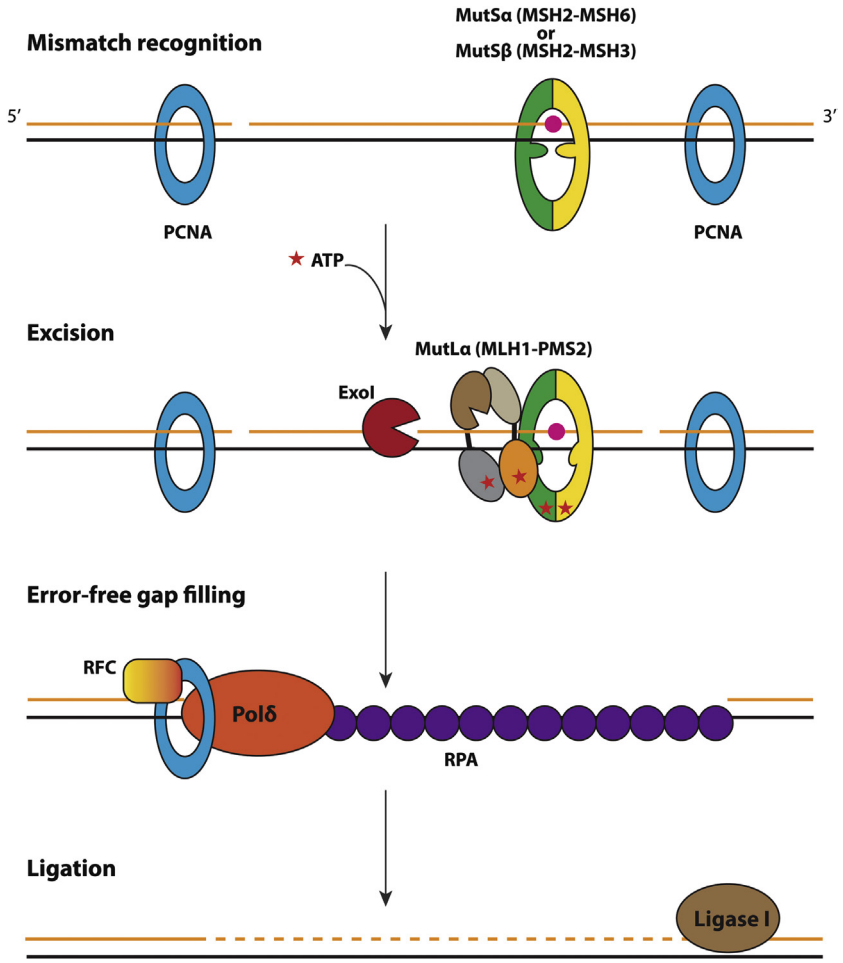

FIGURE 18.1 **Cartoon scheme for MMR in mammalian cells.** MMR is initiated when MutSα (MSH2–MSH6) or MutSβ (MSH2–MSH3) recognizes a mismatch in newly replicated DNA and forms a clamp structure. Nucleotide binding by MutSα or MutSβ induces a conformational switch allowing the recruitment of MutLα (MLH1–PMS2). MutSα or MutSβ can assume a sliding-clamp conformation. PCNA facilitates the recruitment of MMR proteins to the vicinity of the replication fork via a PIP motif on MSH3 and MSH6. ATP binding and hydrolysis at NBDs in both subunits of MutS and MutL homologs (indicated by *red star*) modulates protein–protein and protein–DNA interactions. Recruitment by MutSα and interaction with PCNA activate a latent endonuclease function in the PMS2 subunit of MutLα that nicks exclusively the newly synthesized strand. The nick provides an entry point for ExoI excision; alternatively, an ExoI-independent pathway requiring MutLα endonuclease activity is utilized (not shown). The resulting single-strand gapped DNA is protected by RPA. Error-free gap filling is carried out by replicative Polδ and DNA ligase I to restore the integrity of the duplex. See text for details.

MutSα, mismatched DNA, and PCNA provides an alternative pathway (see further on), though the molecular details are still being developed [25].

Formation of a single-strand gapped DNA in which the single-strand region is coated with *E. coli* single strand–binding (SSB) protein or eukaryotic replication protein A (RPA) yields a substrate for high-fidelity replicative polymerases, PolIII in *E. coli* or Polδ in eukaryotes. Ligation seals the nick resulting in an intact homoduplex devoid of mismatches. Clamp-like proteins that serve as processivity factors for DNA polymerases, bacterial β-clamp, and eukaryotic PCNA not only facilitate the gap-filling synthesis step, but also have critical albeit incompletely understood roles in recruiting MutS proteins to newly replicated DNA and, in the case of eukaryotes, activating and regulating an endonuclease activity that resides in the PMS2 subunit of MutLα (see further on).

2.2 MutS Homologs

Crystallographic studies of bacterial MutS proteins bearing short C-terminal truncations from *Thermus aquaticus* (Taq) or *E. coli* bound to a mismatched DNA containing a single unpaired T or a G:T mispair, respectively, and ADP provide important insights into MutS function (see Fig. 18.2A) [26–28]. In these structures, the two identical subunits forming the dimer each have five distinct structural domains separated by flexible linkers. domain I at the N-terminus is the MBD; domain II

TABLE 18.1 MMR Factors in *Escherichia coli* and *Homo sapiens*

Escherichia coli	*Homo sapiens*	Function
MutS–MutS	MSH2–MSH6 (MutSα) MSH2–MSH3 (MutSβ)	Mismatch recognition. Heterodimeric MutSα and MutSβ have distinct but overlapping mismatch specificities.
MutL–MutL	MLH1–PMS2 (MutLα)	Molecular matchmaker. *E. coli* MutL activates the MutH endonuclease. Human MutLα possesses an intrinsic endonuclease activity. Participates in excision termination in vitro.
	MLH1–PMS1 (MutLβ)	Unknown
	MLH1–MLH3 (MutLγ)	MutLγ can substitute for MutLα in a minor MMR role, but primary function is in meiotic recombination.
Dam methylase		Promotes N^6-adenine methylation at d(GATC) sites serving as strand discrimination signal in *E. coli*
MutH		Strand-specific endonuclease, nicks daughter strand
UvrD		DNA helicase II, promote excision reaction
RecJ, ExoVII		5′–3′-ssDNA exonuclease
ExoI, ExoVII, ExoX		3′–5′-ssDNA exonuclease
	ExoI	5′–3′-dsDNA exonuclease
β-Clamp	PCNA	DNA polymerase processivity factor; multiple MMR functions
γ-Complex	RFC	Loading of β-clamp/PCNA
SSB	RPA1–3	ssDNA-binding protein
DNA Pol III	Polδ	Replicative DNA polymerase that does gap filling
DNA ligase	Ligase I	Seal nicks after DNA resynthesis
	HMGB1	Accessory protein; stimulates excision

interacts with a second highly conserved MMR protein, MutL (see further on); domain III lies between the MBD and the NBD located in domain V. Long α helices or lever arms in domains III and IV propagate conformational changes between the MBD and NBDs of MutS that are separated by approximately 70Å. The two composite NBDs are members of the ABC (ATP-binding cassette) ATPase superfamily and are each comprised of residues from both subunits. They reside at the primary dimerization interface in domain V that also contains a conserved helix-turn-helix motif that promotes dimerization (see Ref. [29]). The structure of the short C-terminus that was deleted in earlier structural studies is essential for MMR at physiological levels of protein and may stabilize the dimer as well as help confer asymmetry of the NBDs as shown for hMutSβ (see further on Ref. [30]).

When bound to a mismatched DNA, MutS is a clamp in which the two previously identical subunits now exhibit asymmetry as only one MBD directly contacts the mismatched base, while the other MBD makes largely van der Waal and hydrogen bond contacts with flanking DNA. This structural and functional asymmetry in the bacterial proteins presages the heterodimeric nature of eukaryotic MMR proteins (see later). The DNA is sharply kinked at the mismatch by about 60° with widening of the minor groove at the mismatch to accommodate the MBD and corresponding narrowing of the opposing major groove.

Phe39 in a conserved Phe-X-Glu motif in domain I of Taq MutS was presumed to be in close proximity to the mismatched base, based on cross-linking studies of Taq MutS bound to a mismatch DNA containing a 5-iododeoxyuridine cross-linking moiety [29]. Mutation to alanine in the related *E. coli* MutS protein abolished mismatched DNA binding in vitro. The crystal structures confirmed that this Phe residue in one of the subunits approaches from the minor groove of the heteroduplex DNA and stacks with the unpaired base extruding it into the minor groove. A hydrogen bond between a carboxyl oxygen of a conserved Glu residue in the same subunit and the mismatched base is also observed [31]. Genetic and biochemical studies confirm that these two residues are essential for proper mismatch recognition in MutS and MutSα, but are notably absent in MutSβ (reviewed in Refs. [11,29]).

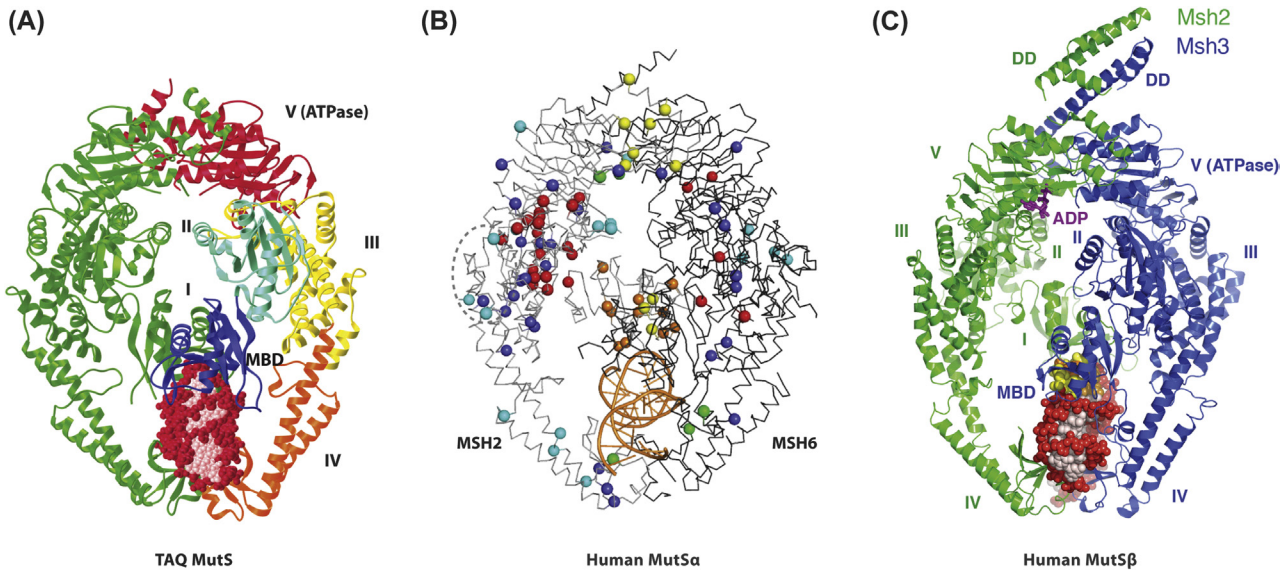

FIGURE 18.2 **Structural models for MutS homologs.** (A) *Thermus aquaticus* MutS bound to a +1IDL mismatched DNA (1EWQ.pdb). The two protein monomers are represented by ribbon diagrams. The DNA is shown in a space-filling model, in which the *backbone atoms are red* and *bases are pink*. In the A subunit, the five structural domains are colored—domain I (mispair-binding domain, MBD) is *blue*; II (connector domain) is *cyan*; III is *yellow*; IV is *pink*; V (nucleotide-binding domain, NBD) is *red*. The B subunit is *green (Reproduced with permission from Yang W, Junop MS, Ban C, Obmolova G, Hsieh P. DNA mismatch repair: from structure to mechanism. Cold Spring Harb Symp Quant Biol 2000;65:225–32)*. (B) Structural model for human MutSα with map of Lynch syndrome mutations (2O8B.pdb). MSH2 and MSh6 are shown as *light and dark gray* Cα chain traces, respectively. Mismatched G:T DNA is *orange*. Lynch syndrome (HNPCC) alleles are indicated by colored dots reflecting hypothetical function. *Cyan*—protein–protein interactions; *blue*—protein stability; *red*—stability/allostery; *yellow*—MSH2–MSH6 interface; *green*—nucleotide-binding sites *(Reproduced with permission from Warren JJ, Pohlhaus TJ, Changela A, Iyer RR, Modrich PL, Beese LS. Structure of the human MutSα DNA lesion recognition complex. Mol Cell 2007;26(4):579–92)*. (C) Ribbon diagram of the structure of human MutSβ, with *MSH2 in green* and *MSH3 in blue* bound to a +3IDL (3THY.pdb). The DNA is shown in a space-filling model with the backbone in *red*, bases in *light pink*, and the unpaired nucleotides in *yellow and orange*. ADP bound to MSH2 is shown in *purple sticks (Reproduced with permission from Gupta S, Gellert M, Yang W. Mechanism of mismatch recognition revealed by human MutSβ bound to unpaired DNA loops. Nat Struct Mol Biol 2012;19(1):72–8)*.

To a first approximation, the crystal structure of a hMutSα-ADP-G:T mismatch complex, comprising full-length MSH2 and a truncated MSH6 missing the first 340 residues, resembles the bacterial MutS structures [32]. Thus, MutSα forms a protein clamp with two channels, the larger one accommodating the kinked mismatched DNA (see Fig. 18.2B). Both MSH2 and MSH6 have five structured domains with the MBD and two composite NBDs containing the ABC ATPase motif at opposing ends of the molecule. Subunit asymmetry evident in the bacterial MutS structures is recapitulated in hMutSα. MSH6, containing the Phe-X-Glu motif (Phe432), contacts the mismatched base, while MSH2 contacts the flanking DNA accompanied by a 45° kink at the mismatch. Lynch syndrome alleles map to virtually all regions of hMutSα. While MSH2 is more or less colinear with bacterial MutS proteins, MSH6 and MSH3 have an additional N-terminal extension of several hundred amino acids in which resides the PCNA-interacting protein (PIP) motif that mediates physical association of MutSα and MutSβ with the processivity factor for replicative polymerases. This N-terminal domain has been deleted in the crystal structures of hMutSα and hMutSβ to aid in structural determination. However, small-angle X-ray scattering (SAXS) of yeast and human MutSα and hMutSβ provides information about the MutS homolog–PCNA interaction and reveals differences in the structure of this N-terminal extension that is disordered in yeast but a globular domain in human MutS homologs [33,34]. In addition, MSH6, but not MSH2 or MSH3, contains a PWWP domain that interacts with histone methylation marks (see further on).

The absence of a Phe-X-Glu mismatch-binding motif in MSH3 and the preference of MutSβ for both small and larger IDLs raise questions about how mismatch recognition is carried out by MutSβ. In addition to mismatch specificity and the architecture of the MBDs (see later), MutSα and MutSβ differ in other respects, for example, nucleotide-induced conformational changes and the ability of MutSα to interact simultaneously with MutLα and PCNA whereas PCNA and MutLα compete for binding to MutSβ [35]. *In vivo* studies of MMR in *S. cerevisiae* reveal that mismatch recognition differs in significant ways between Msh2–Msh3 and Msh2–Msh6 and suggest that mismatch recognition by Msh2–Msh3 requires DNA bending and strand separation at the mismatch using residues in the MBDs of both Msh2 and Msh3 [36–38].

An ensemble of crystal structures of a trimmed human MutSβ bound to IDLs of 2, 3, 4, or 6 nt and one ADP confirms the bending and strand separation model [39]. MutSβ injects a conserved tyrosine–lysine pair into the IDL site that, together with residues chiefly from the MBD of MSH3, but also from MSH2 in the case of larger IDLs, distort the sugar–phosphate backbone of IDLs to achieve sharp substrate bending and strand separation at the IDL (Fig. 18.2C). Importantly, MutSβ can accommodate IDLs of varying length by modulating the degree of DNA bending and allowing domains IV of MSH2 and MSH3 to move independently. The structural data in conjunction with extensive genetic and biochemical studies provide new insights into the roles of these proteins outside post-replication repair (reviewed in Ref. [12]). Single molecule approaches, such as atomic force microscopy (AFM), FRET, and SAXS are providing new insights into mismatch recognition (see Ref. [40]). DNA flexibility and base stacking influence recognition, as the propensity of a DNA lesion to bend and deform regulates access to the MBD [41–43].

The highly conserved composite ATP-binding sites of MutS are critical for MMR, as nucleotide binding regulates the interaction of MutS on DNA and its interaction with MutL and other proteins. The asymmetry of the two MutS subunits induced by mismatch binding is further elaborated by nucleotide-induced conformational changes; nucleotide binding and hydrolysis promote allosteric regulation as opposed to fueling a protein machine like a helicase. Because of its central role, much effort has been focused on understanding the role of ATP binding and hydrolysis in the context of MMR with multiple models under consideration at various times. See reviews in 2010s, as only a small portion of the original literature is cited here [11,12,44].

The structure of hMutSβ, including the extreme C-terminus that is absent in the bacterial MutS structures, defines the full dimerization domain of MutS proteins and provides a structural basis for the intimate connection between subunit dimerization and the NBDs. Several α helices from MSH2 and MSH3, including a previously described helix-turn-helix (HTH) domain found in all MutS proteins form a hydrophobic bundle that stabilizes the dimer [39,45]. The composite nature of the NBDs in which the N1, N3, and N4 nucleotide-binding motifs derive from one subunit, while the N2 motif is contributed by the other subunit underlie a complex structural arrangement in which nucleotide occupancy in one subunit can influence the ATP-binding site of the other. This is mediated by the dimerization domains that facilitate communication between the two NBDs. Thus, the HTH motif in the dimerization domain contacts the nucleotide-binding site directly through a conserved trio of amino acids and also interacts with the N2 nucleotide-binding motif contributed in trans by the partnering subunit. The MutSβ structural data also reveal for the first time motifs in the NBD that can bloc an MSH3 ATP-binding site providing a molecular framework for regulating asymmetric nucleotide binding and exchange regulated by mismatch binding [39].

The inherent asymmetry of the two protein subunits observed in the MBD is mirrored in the NBDs of MutS proteins (see Ref. [11]). Thus, MSH3 and MSH6 are more active ATPases than MSH2 in free MutSα and MutSβ. Biochemical, structural, and genetic experiments reveal that the NBDs of *E. coli* MutS and yeast and human Msh2 and Msh6 bind ATP with different affinities and kinetics and that nucleotide occupancy in one subunit influences the ATP-binding site of the partnering subunit [46–53]. In particular, mismatch binding strongly inhibits the ATPase activity of MSH3 and MSH6, but only weakly affects MSH2. Mismatch binding is correlated with broad movement of MSH3 and MSH6 domains leading to their intimate association, whereas the domains in MSH2 remain loosely associated. Collectively, genetic, biochemical, and structural data lead Gupta et al. to suggest that binding to a mismatch induces a conformational change in the ATPase domain and dimerization domain. Nucleotide binding is blocked in MSH3 and MSH6 but allowed in MSH2 resulting in a conformation that recruits MutLα and licenses MMR [39]. These structural studies provide a platform for testing molecular mechanism.

2.3 MutL Homologs

Less is known about how MutL proteins function compared to MutS homologs, but the endonuclease activity of most MutL proteins clearly plays a pivotal role in MMR and possibly other cellular functions as well. Thus, understanding how this class of MMR proteins works is paramount. Prokaryotic MutL proteins and eukaryotic MutL homologs are homodimers and heterodimers, respectively (reviewed in Ref. [54,55]). They belong to the GHKL (Gyrase b, Hsp90, Histidine kinases, and MutL homologs) superfamily of ATPases [56]. MutL proteins have a conserved N-terminal domain in which resides the four conserved motifs of the GHKL ATPase NBD and a C-terminal dimerization domain. The endonuclease activity found in some, but not all, MutL homologs [25], is located in the C-terminal domain (CTD). Separating these two domains is a flexible linker of varying lengths (see Fig. 18.3). The ATPase activity of MutL proteins is absolutely required for MMR in vivo; correspondingly, Lynch syndrome mutations cluster in this region. The recurring theme of nonequivalent subunits in the key MMR proteins is readily apparent in MutL proteins. Numerous studies reveal that "equivalent" point substitution mutations in the NBDs of MLH1 and PMS2 (or Pms1) do not yield equivalent phenotypes in vivo or biochemical properties

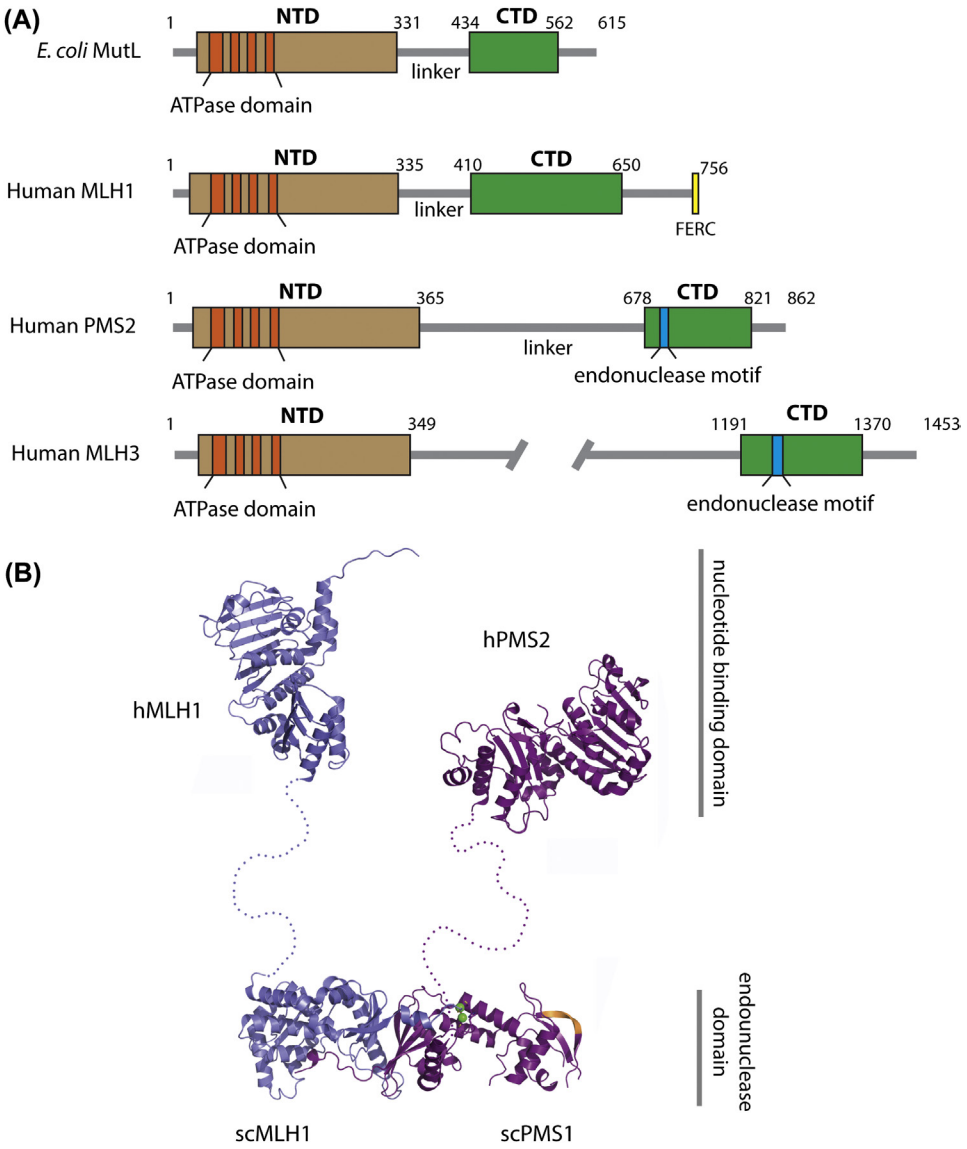

FIGURE 18.3 Architecture of MutL homologs. (A) Structural domains of *E. coli* MutL and human MutL homologs. The N-terminal domain (NTD) is indicated by a *tan box*, the C-terminal domain by a *green box*. The ATPase domain in the NTD consists of four highly conserved motifs (shown in *orange*). The endonuclease domain of PMS2 and MLH3 are in *blue*. The *yellow box* is the conserved FERC sequence of MLH1 (see text). (B) A hypothetical composite model for a eukaryotic MutLα (MLH1–PMS1/PMS2) based on available structures for human NTDs from MLH1 (PDB 4P7A) and PMS2 (PDB 1H7S) [59] shown as ribbon models and CTDs (endonuclease domain) from *S. cerevisiae* MLH1–PMS1 (PDB 4E4W) shown as ribbon models [24]. MLH1 is *blue*, and PMS2/PMS1 is *purple*. Green denotes two zinc atoms in the endonuclease active site. A putative PCNA interaction motif in PMS1 based on *B. subtilis* MutL is shown in *orange*. *Dotted line* represents the unstructured linker domain.

in vitro (see Ref. [54]). In eukaryotes, several MutL homologs exist that form heterodimers with sometimes overlapping but distinct functions. MutLα (MLH1–PMS2) or the equivalent scMlh1–Pms1 is the major MMR protein. Mutation of MLH1 accounts for a large fraction of Lynch syndrome alleles with a much smaller number attributable to loss of PMS2, and epigenetic silencing of the *MLH1* promoter at CpG islands occurs in spontaneous tumors. MutLβ (MLH1–PMS1) or scMlh1–Mlh2 is not thought to have a significant role in MMR, although data in 2014 on yeast suggest that it might act as an accessory factor [57]. Its role in human MMR, if any, is unknown. A third MutL protein, MLH1–MHL3, MutLγ, is important for meiotic recombination where it interacts with a meiotic-specific MutS homolog, Msh4–Msh5 (see Ref. [12]). It has a minor role in the repair of IDLs in yeast, and based on the cancer susceptibility and mutator phenotypes of knockout *MLH3* mice [58], probably contributes to MMR in mammals as well.

The N-terminal domain, about 330 residues, is multifunctional. In addition to the ATPase domain, it is responsible for both DNA binding and for interactions between MutL proteins and other repair partners including MutS proteins and PCNA.

Crystal structures have been solved for *E. coli* MutL–AMPPnP [56], hMLH1–ATP [59], hPMS2–ATPγS [60], and scPMS1–AMPPnP [61]. Structural and biochemical studies of the N-terminal domain from *E. coli* MutL reveal that ATP binding causes dimerization of the N-terminal domain with large numbers of conformational changes induced by a cycle of ATP binding, hydrolysis, and ADP release [60]. The structure also identifies a conserved DNA-binding groove, and establishes that binding to DNA stimulates the otherwise modest ATPase activity of MutL with attendant conformational changes. One consequence of ATP binding by MutL proteins is their conversion from open proteins to ring-like structures that have been shown to bind DNA (see later). AFM of yeast and human MutLα define at least four distinct conformations modulated by nucleotide binding, hydrolysis, and ADP release [62]. The proline-rich linker is poorly conserved but is thought to help mediate these large, asymmetric conformational changes. A working model based on these and other studies is that binding of ATP by MutL proteins results in dimerization of the N-terminal domains creating a DNA-binding groove that when occupied, results in a semicondensed state (reviewed in Ref. [54]). Efforts to relate these structural changes to MutL function are underway.

The CTD, about 200 residues, is the primary dimerization interface of MutL proteins. In prokaryotes with no MutH and in eukaryotes, the CTD of MutL or scPms1 (hPMS2) and Mlh3 harbors a conserved $DQHA(X)_2E(X)_4E$ endonuclease motif as well as other conserved motifs that together constitute the endonuclease domain [25,55,63–65]. The CTD of MutLα also harbors the interaction domain with Exo1 [24,66]. Despite low sequence homology, the available structural information from CTDs of *E. coli*, *Bacillus subtilis*, and *S. cerevisiae* suggests overall conservation of topology [24,67,68]. In the structure of the CTD from scMutLα, each CTD is composed of distinct dimerization and regulatory domains [24]. The dimer interface between Mlh1 and Pms1 is extensive. The regulatory domain of Mlh1 contains an MIP-box motif that mediates interaction with other proteins including Exo1. The endonuclease site resides in a connector domain positioned at the dimerization interface between Mlh1 and Pms1. Given the importance of the endonuclease activity for MMR, its location at this critical interface is perhaps not surprising. It consists of the expected $DQHA(X)_2E(X)_4E$ motif plus three other motifs that constitute the metal-binding site occupied by two zinc atoms. The C-terminal amino acid of Mlh1, Cys769, part of a conserved FERC motif, interacts with the metal-binding site in the crystal structure. The requirement for Cys769 in MMR in vivo is unclear as conflicting results are obtained in different mutator assays [24,64], and its role in human MutLα remained to be determined.

A longstanding question is the nature of the interaction between MutS and MutL proteins. When is MutL recruited to mismatched DNA in the presence of MutS, how long-lived is the interaction, and what is the stoichiometry of MutS and MutL proteins on mismatched DNA? Both ATP binding and mismatch bonding are required for the interaction of bacterial MutS with MutL and scMsh2–Msh6 with scMlh1–Pms1 ([69] and references cited therein). Hydrogen–deuterium exchange mass spectrometry in the presence of *E. coli* MutS, ATP, a mismatched DNA and *E. coli* MutL identifies a region in *E. coli* MutS "connector" domain II that exhibited decreased solvent accessibility in the presence of MutL [69]. Genetic and biochemical experiments confirm that Q211 or Q212 or both, mediate a MutS–MutL interaction in vitro and are required for MMR in vivo. The residues map to a structurally conserved region in scMsh2, but not scMsh6, and additional experiments confirm that the same region in Msh2, but not Msh6, is required for a MutSα–MutLα interaction in vitro and in vivo. Thus, the functional asymmetry evident in the MBDs of MutS proteins is also evident in the interaction with MutL, and the authors propose that ATP and mismatch recognition by MutS serve to present regions in domain II to MutL. Cross-linking and FRET studies also suggest an interaction between the N-terminal domain of *E. coli* MutL and domain II of only one MutS subunit [70]. In 2015, a crystal structure of the NTD of *E. coli* MutL site specifically cross-linked to *E. coli* MutS with a previously crystallized C-terminal truncation in the presence of AMP-PNP and a G:T mismatched DNA, reveals two interfaces [71]. One involves a region of the ATPase domain of MutL and a repositioned connector domain II of one MutS subunit consistent with deuterium exchange mass spectrometry [72]. The second involves an adjacent region of the ATPase domain of MutL and the ATPase and core domains of the other subunit of MutS including a conserved peptide loop in the core domain implicated in MutS–MutL interactions in *B. subtilis* MutS and human MutSβ [35,73]. Each MutL monomer is interacting with both subunits of the MutS dimer accompanied by large movements in multiple domains detected by FRET that are postulated to reflect an ATP-induced sliding-clamp conformation for MutS. Collectively, these advances highlight questions for further investigation.

2.4 Licensing Targeted Excision

An unresolved question is exactly how recognition of a mismatch by MutS proteins leads to recruitment of MutL proteins and licensing of the downstream excision step. In vitro MMR assays invariably require DNAs with a mismatch and a pre-existing nick even in the presence of MutLα with its latent nicking activity, and MMR-mediated excision is directed to the pre-nicked strand. *E. coli*, an outlier, depends on MutH for strand-specific nicking, but Dam GATC sites that are recognition sites for MutH can be several 1000 bp from a mismatch. MMR occurs on both the leading and lagging strands. In *S. cerevisae*, there is support for strand breaks in the Okazaki fragment-containing lagging strand serving as a strand discrimination

signal, but the leading strand is thought to be comparatively barren of such breaks. It has been proposed that PCNA- and RNase H2-mediated removal of misincorporated ribonucleotides could provide strand breaks for MMR, though genetic data suggest it is a minor pathway (see Refs. [1,12]). In any case, MMR has to solve an action-at-a-distance problem.

Early models invoke a stationary complex of MutS and MutL at the mismatch and DNA bending or looping to bring distant sites together based on in vitro biochemical studies (see Ref. [29]) or ATP-powered translocation by MutS along the DNA to facilitate long-distance communication (reviewed in Ref. [22]). A nucleotide-switch or sliding-clamp model posits that ADP to ATP exchange upon mismatch binding induces conformational changes that convert MutS proteins from a clamp on the mismatch to a diffusing or sliding clamp that migrates along the DNA [74,75]. In this model, iterative rounds of MutS loading at the mismatch can occur leading to multiple MutS–MutL complexes on the DNA. Genetic, biochemical, and biophysical studies support a nucleotide switch in mismatch-bound MutS proteins and the formation of ATP-dependent sliding clamps in vitro, and mutations that disrupt this nucleotide switch disrupt MMR in vivo (reviewed in Refs. [11,12]). The sliding-clamp model is also consistent with in vitro experiments showing that a physical block between the mismatch and a DNA nick inhibits MMR [76,77]. Single-molecule fluorescence studies probe the movement of MutS on DNA and detect a corkscrew-like motion in the presence of ATP accompanied by distinct conformational changes in both the DNA and the protein that might facilitate a mismatch search [62,78–81].

What happens to a MutS–MutL complex? In a study of Q-dot-labeled MutSα and MutLα on λ DNA containing three GT mismatches, MutSα–MutLα complexes in a 1:1 ratio are seen to move along the DNA as sliding clamps [82] (see discussion in Ref. [40]). The final verdict is not in, however, as several studies suggest that the association of MutL with MutS, while ATP dependent, does not promote diffusion from the mismatch and involves a biased loading of MutL relative to MutS. A 2015 single-molecule FRET study proposes that Taq MutL traps MutS at the mismatch after MutS binds ATP and undergoes the first of multiple conformational changes [80]. Multiple loading of MutS proteins is observed that is inhibited by MutL, and the stoichiometry is consistent with a small excess of MutL over MutS. Visualization of fluorescently tagged and biologically active *E. coli* MutS and MutL in vivo [83] is also most consistent with colocalization at mismatches of MutS and MutL. MutL is found in several-fold excess over MutS and is thought to reflect multiple loading of MutL on the DNA extending from the mismatch towards a strand discrimination site in a manner possibly related to proposed catalytic loading of MutLα by MutSα in *S. cerevisiae* (see Refs. [12,84]). In reconstituted MMR assays, hMutLα helps to limit the extent of excision such that it terminates just beyond the mismatch suggesting that MutLα supplies mismatch positional information [19]. A requirement for more MutL than MutS might also explain why MutL is limiting for MMR in vivo and is consistent with previous *E. coli* MutS–MutL footprinting experiments (discussed in Refs. [40,83]).

Finally, even the existence of a stable ternary complex of MutS, MutL, and a mismatched DNA, particularly in the case of eukaryotic MMR proteins, is being questioned as studies in the 2010s suggest that the interactions between MutS and MutL may be transient in vivo. Attempts to isolate presumptive ternary complexes have required cross-linking or chemical trapping [70,71], and scMsh2 and scMlh1 foci do not always colocalize in vivo [84]. Furthermore, in vivo and in vitro studies indicate that MutSα and a mismatched DNA are not required to activate the endonuclease activity of MutLα per se, but may have roles in recruiting and/or retaining MutLα and PCNA to newly replicated and mismatched DNA so that PCNA can activate the MutLα endonuclease [84–86]. Much work remains to understand how MutS proteins find rare mismatches in a sea of genomic DNA and recruit MutL to license MMR.

2.5 Strand Discrimination

How is excision directly exclusively to the newly synthesized strand? *E. coli*, almost uniquely, exploits the transient undermethylation of newly synthesized DNA and incision by the MutH methyl-directed endonuclease. For virtually all other organisms, another mechanism(s) must be in play. In the case of the lagging strand containing Okazaki fragments, it is easy to envision EXO1 acting at transient breaks. In the case of the leading strand or in EXO1-independent excision, the mechanism is less obvious. It has been proposed that PCNA dictates the strand bias of MutLα nicking directing incision exclusively to the newly synthesized strand. The latent endonuclease activity of MutLα is activated in vitro by RFC and PCNA and utilizes the PIP motif in the PMS2 subunit for direct interaction. RFC and PCNA, but neither a mismatch nor MutSα, is required to activate MutLα in vitro and direct cleavage to the strand with a preexisting nick [85]. Because RFC loads PCNA with a fixed orientation preferentially at 3′-double-strand–single-strand junctions [87], PCNA serves as a de facto strand–discrimination signal. Perhaps a specific geometry of the MutLα–PCNA interaction imposes a strand bias on MutLα incision, but this remains unproven [88,89]. *In vivo*, the situation is more complicated as PCNA has been shown to bind to DNA structures that have single-strand characteristics, such as a small number of extruded triplet repeats leading, in principle, to error-prone repair if the template strand is indiscriminately nicked by MutLα [90]. Another possibility is that nicks introduced by RNaseH processing of ribonucleotides misincorporated into DNA may serve as a strand discrimination signal (see discussion in Ref. [1]).

However, *S. cerevisiae* strains missing RNaseH2 exhibit only a mild mutator phenotype suggesting that other mechanisms must operate to confer strand specificity (see discussion in Ref. [12]). An unambiguous mechanism for targeting the MutLα endonuclease to newly synthesized strands, particularly in the case of the leading strand, remains elusive.

A further wrinkle in the MMR excision step is the existence of Exo1-independent excision. Inactivation of *EXO1* in *S. cerevisiae* or mice confers only a weak mutator phenotype, and in mouse models fails to recapitulate the mutation or cancer spectrum of mutations in essential MMR genes. Low but detectable levels of MMR are observed in MMR assays using Exo1-deficient mouse cell extracts. A series of genetic tests in *S. cerevisiae* identified mutations that differentially affect Exo1-independent versus Exo1-dependent pathways (see Ref. [86]). In particular, loss of the Pms1 endonuclease activity (equivalent to hPMS2) conferred hypersensitivity in an *exo1Δ* strain as did certain mutations in the gene that encodes PCNA, *pol30* [91]. These results provide support for a critical role for MutLα endonuclease activity in an MMR excision pathway that does not involve Exo1 and suggests that recruitment of MutLα to mismatched DNA by MutSα and the activation of MutLα by PCNA are essential features of this pathway in vivo.

Imaging studies in *S. cerevisiae* identify Msh2–Msh6 foci that colocalize with replication factories. Mlh1–Pms1 foci are dependent on Msh2–Msh6, but they seldom colocalize with Msh2–Msh6 foci or replication machinery [84]. The authors propose a model in which MutSα (or MutSβ) bound to a mismatch catalytically loads multiple molecules of MutLα. Upon interaction with PCNA, these MutLα molecules can incise the newly synthesized strand providing access for Exo1 excising in a 5′–3′-fashion to create a gapped DNA intermediate. In an Exo1-independent scenario, several nonexclusive pathways for excision and processing of a MutLα-nicked heteroduplex may occur: (1) strand displacement synthesis by Polδ from the nick followed by flap cleavage and ligation; (2) additional DNA nicking by Mlh1–Pms1 followed by Polδ-dependent strand displacement and/or gap filling; (3) excision by the 3′–5′-proofreading exonuclease of replicative polymerases. In vitro MMR assays utilizing purified hMutSα, hMutLα, RFC, PCNA, RPA, and DNA Polδ yield no excision intermediates but support synthesis–driven strand displacement by Polδ in this EXO1-independent MMR system [92]. Confirmation in mammalian cells awaits as does a detailed study of the prevalence and kinetics of these pathways.

3. MISMATCH REPAIR AND THE DNA-DAMAGE RESPONSE

3.1 Alkylation Damage and Thiopurines

The MMR system is also implicated in the repair and cytoxicity of a subset of DNA lesions caused by S_N1 DNA alkylators, 6-thioguanine, fluoropyrimidines, cisplatin, UV light, and certain environmental carcinogens that form DNA adducts (reviewed in Refs. [4,29]). The S_N1 DNA alkylators, for example, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), methylnitrosourea, and the chemotherapy drug temozolomide, methylate all four DNA bases producing a variety of potentially cytotoxic lesions. Exposure to these alkylators induces a DNA-damage response resulting in cell cycle arrest and apoptosis that is dependent on MutSα and MutLα MMR proteins. Despite constituting a small fraction of total lesions, O^6-methylguanine (O^6me-G) is the key contributor to the mutagenic and cytotoxic effects of S_N1 alkylators. During replication, polymerases misincorporate opposite O^6me-G forming O^6me-G:T mispairs that, if unrepaired, lead to G to A transition mutations. O^6-methylguanine-DNA methyltransferase (MGMT) directly reverses O^6meG in cells and plays an important role in protecting against cytotoxic effects of S_N1 alkylators and preventing tumor formation in vivo [93]. Thiopurines, used in chemotherapy, are incorporated into DNA and undergo spontaneous methylation by endogenous *S*-adenosylmethionine to form structurally similar 6-thiomethylguanine. Tolerance to thiopurines is also tied to loss of MMR (see Ref. [94]).

First documented in *E. coli*, MMR-deficient mammalian cell lines also exhibit tolerance to alkylating agents and can be almost two orders of magnitude more resistant to killing than comparable MMR-proficient cells (reviewed in Ref. [94]). Low doses of MNNG induce a G_2/M cell cycle arrest in the second cell cycle after exposure that is dependent on MMR proteins (reviewed in Refs. [11,95]). In cells exposed to alkylating agents, ATM and Rad3-related (ATR) kinase undergoes autophosphorylation and, together with other proteins, such as ATR-interacting protein (ATRIP), an obligate ATR partner, Claspin and TopBP1, activates a signaling cascade ultimately leading to G_2/M cell cycle arrest mediated by downstream targets including the Chk1 checkpoint kinase and others (reviewed in Ref. [96]). Apoptosis directed in most cases by phosphorylated p53 also requires MutSα and MutLα. ATP–ATRIP is recruited to regions of ssDNA bound to RPA via an RPA-interacting motif in ATRIP [97]. Thus, persistent excision intermediates of MMR can activate ATR.

An explanation for the requirement of MMR proteins for cell killing by alkylating agents involves MMR processing of O^6me-G:T mispairs that are recognized by MutSα (reviewed in Ref. [22]). As discussed earlier, MMR-directed excision is targeted exclusively to the newly synthesized strand containing thymidine, whereas O^6meG remains in the DNA possibly triggering repeated cycles of MMR excision followed by resynthesis. Repeated rounds of excision could lead to DSBs. Aberrant or abortive MMR processing at sites of damage can lead to the accumulation of single-strand gaps visualized

by EM [98]. Activation of ATR can occur when ATP–ATRIP is recruited to these regions of ssDNA bound to RPA. These single-strand intermediates that fail to engage the DNA synthesis machinery in the final step of MMR will give rise to broken chromosomes and damage signaling in the next round of replication explaining the delayed-damage response in cells exposed to alkylating agents. Ectopic expression of nuclease-dead EXO1 in mouse embryo fibroblasts in which endogenous EXO1 is absent restores a MSH2–CHK1 interaction and MNNG sensitivity providing support for the role of EXO1-mediated excision in a DNA-damage response [99].

An alternative model that remains to be proven involves direct recruitment of ATR to sites of damage by the MMR machinery (see Ref. [100]). MutSα and MutLα associate with ATR and other damage-signaling proteins, such as TopBP1 and Chk1 in multiprotein complexes in human cells [101,102]. In addition, MutSα and MutLα are required to recruit and activate ATR in the presence of O^6me-G:T-containing DNAs in an in vitro assay scoring for phosphorylation of Chk1 [103], and recruitment of ATR to sites of cisplatin damage is dependent on hMSH2 but not on RPA, Rad17, or the 9-1-1 complex [102].

3.2 Fluorouracil

Fluoropyrimidines, such as 5-fluorouracil (FU) are widely used in chemotherapy and evince a cytotoxic response that is dependent, in part, on MutSα and MutLα (reviewed in Ref. [104]). When FU is metabolized, thymidylate synthase, a key enzyme in de novo pyrimidine biosynthesis, is inhibited resulting in imbalances in nucleotide precursor pools and the incorporation of uracil and fluorouracil in DNA and RNA. Several lines of evidence indicate that incorporation of FdU into DNA is the primary pathway for cell killing. MutSα targets rare dFU:G mispairs resulting in the activation of MutSα ATPase activity [105]. Base excision repair (BER) also targets dFU (see Ref. [93]), and there is evidence that both BER and MMR contribute to the damage response and promote cell killing (see Refs. [106,107]).

3.3 Oxidative Damage and Noncanonical MMR

BER is the primary repair pathway for oxidative DNA damage in which specific glycosylase enzymes remove the damaged base followed by cleavage at the abasic site and gap repair. However, 7,8-dihydro-8-oxo-guanine (8-oxoG) templates 8-oxoG:A mispairs that often escape proofreading due to near normal geometry and are recognized by MutSα (see Refs. [1,11]). Interestingly, MutSα is implicated in a noncanonical MMR pathway that operates largely outside of S-phase in which MutSα recognizes clustered oxidative lesions leading to excision and monoubiquitination of PCNA [108]. This PCNA modification signals an error-prone polymerase, Polη, that carries out the gap-filling step in place of high-fidelity polymerases. Thus, MutSα can be recruited for a mutagenic process. A similar noncanonical MMR pathway dependent on MutSα, MutLα, monoubiquitinated PCNA, and Polη responds to S_N1-type alkylating agents in a variety of cell types and may explain the mutagenicity of alkylating agents (see Ref. [10]). In fact, mutagenic repair involving MMR may be more prevalent than previously thought. MMR-induced mutations are found flanking naturally occurring mismatches [109].

3.4 UV, Cisplatin, and DNA Cross-Links

UV causes cyclobutane pyrimidine dimers (CPDs) and genotoxic (6-4) pyrimidine pyrimidone dimers (6-4 PP) in DNA. Although nucleotide excision repair is the primary repair pathway, *msh2*$^{-/-}$ mice exhibit an increased incidence of UV-induced skin tumors. Mammalian MSH2 in murine and human cells is implicated in cell cycle arrest and apoptosis induced by UV and its loss with increased mutagenesis (reviewed in Ref. [100]). In vitro, MutSα can bind to mismatched CPDs and 6-4 PP. A novel pathway for MMR dependent, UV-induced mutagenesis, and DNA-damage signaling termed "post-TLS repair" invokes prior action at sites of UV damage by error-prone translesion synthesis (TLS) DNA polymerases that synthesize past the UV lesion residing in the template strand but introduce errors [110]. MutSα recognizes the mismatch products of TLS and initiates excision. If the single-strand gaps are not repaired, checkpoint induction occurs, but error-free filling of the gaps mitigates UVC mutagenicity. Deducing an explanation for organ tropism of tumors is oftentimes challenging. In Lynch syndrome, the rate of cellular proliferation is probably an important contributor, but it is unlikely to be the only one. Tsaalbi-Shtylik et al. suggest that loss of post-TLS repair and attendant elevated mutagenesis leads to disruption of multiple tumor-suppressing functions, and in combination with constant exposure to intestinal genotoxins, may explain the colorectal tropism of Lynch syndrome [110].

Cisplatin, a common chemotherapeutic drug, introduces intrastrand and lethal interstrand DNA cross-links (ICLs). There is a large literature on the effects of MMR on survival in cells treated with cisplatin with variable results (reviewed in Ref. [100]). MMR is unlikely to directly remove the cross-link; instead, multiple protein complexes involved in the Fanconi anemia pathway, homologous recombination, DSB repair, and NER converge on the ICL. MMR's role may be

as a modulator of recombination or activity of the Fanconi anemia proteins. A FANCJ–MLH1 interaction suppresses MSH2 activity to promote restart at stalled replication forks [111,112], and MLH1 and PMS2 have been implicated in a p73-dependent apoptotic response to cisplatin [113] indicative of a pleiotropic role for MMR. MMR may modulate other repair pathways that target bulky DNA adducts formed by several environmental carcinogens, for example, benzo[c]phenanthrene dihydrodiol epoxide that modifies adenine residues or benzo[a]pyrene, a polycyclic aromatic hydrocarbon (see Refs. [4,100]).

4. REGULATION OF MMR

Spatiotemporal regulation of MMR is best exemplified by the close association of MMR with replication that confers several advantages. MMR can proceed efficiently, can utilize a transient open state of chromatin at the replication fork, and can collaborate with the replication and MMR machinery including PCNA, RPA, RFC, and replicases (reviewed in Ref. [1]). Correspondingly, expression of MMR genes is highest during S-phase though the increase is modest. Genome-wide assessments of mutational spectra in *S. cerevisiae* strains harboring mutations in MMR genes and/or replicases reveal that MMR is influenced by the replicase, leading versus lagging strand, mismatch composition and local sequence context. MMR corrects errors made by all three replicative polymerases.

How are MMR proteins recruited to newly synthesized DNA? Evidence from bacteria, yeast, and human cells point to a recruitment role for polymerase processivity factors like PCNA. Live cell imaging in *B. subtilis* and *S. cerevisiae* is revealing new details (reviewed in Ref. [12]). In *S. cerevisiae*, Msh2–Msh6 foci in S-phase colocalize with DNA polymerases, PCNA, and RPA; disruption of PCNA binding, for example, by mutating the PIP motif of Msh6 results in loss of the foci [84], and temporal coupling between MMR and replication in yeast is observed [114]. Similar interactions between *B. subtilis* and *E. coli* β-clamps and MutS have been reported (eg, Ref. [115]). The situation is likely more complicated, however, as loss of PCNA–Msh6 interactions in yeast only causes a partial loss of MMR in vivo supporting PCNA-independent pathways. Mlh1–Pms1 foci do not always colocalize with Msh2–Msh6 foci, consistent with the foci representing different MMR intermediates or events. Human MSH6 and MSH3 retain a PIP motif, and both MutSα and MutSβ interact with PCNA in vitro [116]. Furthermore, PCNA is an obligate partner in in vitro 5′-nick directed and bidirectional MMR assays utilizing human proteins and is required to activate the latent endonuclease activity of MutLα discussed previously [18–20,25,88]. Recently, Li and colleagues have reported that an epigenetic histone mark, trimethylation of histone H3K36, recruits MutSα to chromatin utilizing a PWWP recognition domain in MSH6 [117]. Epigenetic modification may serve as a general recruitment tool for MMR though MSH3 lacks a PWWP domain suggesting that other recruitment mechanisms exist.

Epigenetic marks on chromatin and chromatin architecture can modulate MMR. As discussed earlier, histone methylation at H3K36me3 may serve in general recruitment of MutSα to chromatin, an idea that is supported by the presence of MSI in SETD2 methylase-deficient cells [117]. Histone H3 acetylation is also suggested to modulate MMR [118]. Elements of chromatin structure can also inhibit MMR since DNA wrapped around nucleosome cores is generally less accessible, and nucleosomes block excision in in vitro systems (reviewed in Ref. [119]). Chromatin assembly factor 1 (CAF-1) promotes the assembly of nucleosomes on newly replicated DNA and protects lagging strands from excessive degradation in a reconstituted MMR system [120]. Thus, the coordination of MMR with nucleosome reassembly post-replication is critical. MMR delays nucleosome reassembly in vitro, and intriguingly, two key MMR players, MutSα and PCNA, interact with CAF-1 [121].

MMR can be inhibited by targeted degradation of MMR proteins by ubiquitin proteasomes. Histone deacetylase 6 (HDAC6) sequentially deacetylates and ubiquitinates MSH2 leading to the loss of MutSα and MutSβ in human cells [122]. Loss of MSH2 via an ubiquitination-dependent pathway also occurs in a subset of acute lymphoblastoid leukemia (ALL) cells that harbor inactivating chromosomal deletions in at least one of four genes that inhibit an MSH2 degradation pathway [123]. These primary ALL cells have low levels of MSH2 and exhibit MSI, and the loss of MMR may explain clinical tolerance to thiopurine therapy in this patient subpopulation. Another pathway for shutting off MMR is its indirect downregulation by the epidermal growth factor receptor (EGFR), a transmembrane receptor protein kinase that promotes cell growth, tumor progression, and metastasis. Following import into the nucleus, EGFR induces phosphorylation of PCNA at Y211 crippling its interaction with MMR proteins and inhibiting MMR [124]. Regulating MMR levels can also occur through changes in expression of microRNAs (miRs) that respond to DNA damage, with miR-422a, miR-21, and miR-155 being likely candidates ([125] and references cited therein).

5. FUTURE DIRECTIONS

The ubiquitous MMR system has been the focus of much attention in recent years as a critical player in genome stability and tumor suppression and as an important participant in numerous other diverse cellular processes. With respect to repair,

a number of important questions remain. How is the MMR machinery recruited to newly replicated DNA and how is it positioned with respect to the advancing replisome? When do MutS and MutL proteins interact and when do they function separately? Exactly how is the MutL endonuclease activity targeted to the newly synthesized strand, and what is its biological scope? What is the mechanism of recruitment of replicative polymerases to single-strand gaps? In what contexts are error-prone polymerases employed instead and what are the consequences? How is MMR influenced by the higher-order architecture of chromatin and the nucleus? Finally, how can knowledge of MMR mechanism improve clinical diagnostics and therapeutic outcomes? The chapter on MMR is still being written.

GLOSSARY

Apoptosis It is also known as programmed cell death. A highly regulated and coordinated process that results in cell death preceded by characteristic changes including nuclear fragmentation, chromosomal DNA fragmentation, and mRNA decay. Apoptosis is part of normal developmental and differentiation processes and can also be triggered by DNA damage that blocks replication.

DNA excision repair Highly conserved molecular pathways that restore genome integrity after DNA damage by both endogenous and exogenous sources. Three pathways, base excision repair, nucleotide excision repair, and mismatch repair target distinct types of damage including oxidized or alkylated bases, UV photoproducts, and base mispairs by excising or enzymatically removing the damaged or incorrect bases and restoring the correct sequence using DNA polymerases and the undamaged strand as a template for correction. The 2015 Nobel Prize in Chemistry was awarded in recognition of basic advances in our understanding of these three excision repair pathways.

Epigenetic silencing Turning off gene expression by external or environmental factors such as DNA methylation at promoter sequences that inhibits transcription in contrast to changes in nucleotide sequence.

FRET Förster resonance energy transfer describes energy transfer between two light-sensitive molecules or chromophores, a donor and an acceptor. FRET is very sensitive to small changes in distance and is used to measure association/dissociation events and conformational changes in biological molecules bearing precisely positioned chromophores.

Indels The insertion or deletion of bases in the genome of an organism that accumulate in the absence of DNA mismatch repair; indels occur more frequently in microsatellite regions. In coding regions, indels that are not multiples of three will result in a frameshift mutation.

Microsatellite instability Contraction or expansion of a genomic region caused by loss of mismatch repair commonly in a region of mono- or dinucleotide repeats; its presence is strongly correlated with Lynch syndrome colorectal cancer.

Mutator phenotype Phenomenon whereby an organism exhibits a greatly elevated rate of spontaneous mutation, usually genome-wide, due to the genetic inactivation of a protective pathway (eg, DNA mismatch repair).

Posttranslational modification Covalent modification of proteins (eg, phosphorylation, acetylation, or ubiquitination), usually involving specialized enzymes, that occurs during or after protein synthesis by translating ribosomes on mRNA. Such modifications regulate many aspects of protein function, stability, and cellular localization.

Ubiquitin-mediated proteolysis In eukaryotes, the selective breakdown of proteins by a proteasome complex in response to the covalent addition of a small, 8.5 kDa regulatory protein, ubiquitin, by a group of ubiquitin-activating/conjugating/ligase enzymes.

LIST OF ABBREVIATIONS

6-4PP (6-4)Pyrimidine pyrimidone dimers
8-oxo-G 7,8-Dihydro-8-oxo-guanine
ATR ATM and Rad3 related
ATRIP TR-interacting protein
BER Base excision repair
CPDs Cyclobutane pyrimidine
CTD C-terminal domain
DSB Double-strand break
EGFR Epidermal growth factor receptor
FRET Förster resonance energy transfer
GHKL Gyrase b, Hsp90, histidine kinases and MutL homologs
HTH Helix-turn-helix
ICLs Interstrand cross-links
IDL Insertion/deletion loop
MBD Mismatch-binding domain of MutS
MGMT O^6-methylguanine-DNA methyltransferase
MIP MLH1-interacting protein
miR micro-RNA
MLH MutL homolog
MMR Mismatch repair
MNNG *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine

MSH MutS homolog
MSI Microsatellite instability
NBD Nucleotide-binding domain
NTD N-terminal domain
***O*^{6}me-G** *O*^{6}-methylguanine
SAXS Small-angle X-ray scattering
TLS Translesion synthesis

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