# DNA Mismatch Repair: Functions and Mechanisms

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## 1. Introduction

### 1.1. Mutation, Mismatch Repair, and Replication Fidelity

Mutations are rare events, occurring spontaneously at a frequency of 1 per $10^9-10^{10}$ base pairs per cell division.[1,2] Nucleotide misincorporation during DNA synthesis yields noncomplementary base pairs or mismatches within the DNA helix, which if uncorrected are fixed as mutations during the subsequent round of DNA replication. Mutations can also arise via incorporation of chemically damaged nucleotides or by incorporation of an undamaged nucleotide opposite a damaged base within the template strand.[3-5] Strand slippage or formation of unusual secondary structures within DNA, especially within repetitive sequences, can also result in mutations when processed aberrantly during replication, recombination, or repair.[6-9]

Base pair geometry and the nature of the DNA polymerase involved result in an error rate of $10^{-4}-10^{-5}$ at the nucleotide insertion step of DNA synthesis.[10] In the event of incorrect insertion, the proofreading exonuclease associated with some DNA polymerases edits the mistake, permitting the enzyme to make a second attempt at correct synthesis. Nucleotide selection and editing in this manner confers an error rate of $\sim10^{-7}$ per bp per replication. Mistakes that escape these fidelity devices are corrected by mismatch repair, further elevating fidelity 50−1000-fold. In this pathway, a noncanonical base pair is recognized by a MutS homologue, which in conjunction with a MutL homologue initiates replacement of the offending nucleotide on the newly synthesized strand by an excision repair mechanism. The activities that participate in this process have been best characterized in *Escherichia coli*, although substantial information is now available on the yeast and human systems.

In addition to replication errors, mismatches arise as a natural consequence of genetic recombination when the heteroduplex intermediate spans genetic differences between the recombining helices, and such mispairs can be processed by the repair system.[11,12] A variety of base pair anomalies resulting from DNA damage are also subject to processing by mismatch repair. These include base pairs containing $O^6$-methylguanine,[13-17] 8-oxoguanine,[18,19] carcinogen adducts,[20] UV photo products,[21-23] and cisplatin adducts.[16,24,25]

### 1.2. Biological Significance of Mismatch Repair

Genetic inactivation of the mismatch repair system elevates spontaneous mutability 50−1000-fold.[26-31] Mismatch repair defects lead to highly elevated rates of base substitution and frameshift mutations, permit illegitimate recombination between quasi-homologous sequences,[32,33] and render mam-

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malian cells resistant to the cytotoxic effects of several classes of DNA damaging agents.[27,34−36] Inactivation of the human mismatch repair system is the cause of hereditary nonpolyposis colon cancer (HNPCC)[37−40] and has been implicated in the development of a subset of sporadic tumors that occur in a variety of tissues.[39−43]

## 2. *Escherichia coli* Methyl-Directed Mismatch Repair

The notion that mismatches generated during DNA transactions might provoke their own repair was initially suggested by Holliday[44] and Whitehouse[45] to account for marker effects associated with meiotic recombination. On the basis of the low transformation efficiency of certain genetic markers into *Streptococcus pneumoniae*, Ephrussi-Taylor and colleagues proposed a mismatch rectification process in this bacterium that was targeted to the incoming DNA strand.[46] Direct proof that mismatches can provoke their own repair was provided by Meselson and colleagues who transfected *E. coli* with phage λ heteroduplex DNAs containing one or more mismatched base pairs.[47,48] These experiments showed that different mismatches can be rectified with differing efficiencies, implying that rectification is dependent on mismatch recognition. They also demonstrated that co-repair of closely linked mismatches usually occurs on the same DNA strand, an effect that was interpreted in terms of an excision mode of repair with a tract size of several thousand nucleotides.

### 2.1. Strand Discrimination and Mismatch Specificity

Although early studies of mismatch repair were prompted by an interest in recombination marker effects, Wagner and Meselson postulated that mismatch repair could also contribute to replication fidelity provided that the reaction could be directed to the newly synthesized DNA strand.[48] They suggested that this could be accomplished by exploitation of secondary signals within the helix such as the transient

absence of methylation on newly synthesized DNA or via a "special relation to the replication complex".[48] Methyl direction was confirmed in *E. coli* when heteroduplex repair was shown to be controlled by the status of adenine modification at GATC sequences.[49] Newly synthesized DNA is subject to modification at this sequence by the Dam methylase after a transient delay.[50−52] Mismatch repair of hemi-methylated DNA occurs on the unmodified strand, heteroduplex DNA lacking the Dam modification on either strand is also processed but with little or no strand bias, and DNA that is modified on both strands is not repaired.[49,53] As expected from such observations, *E. coli* cells deficient in Dam methylase are mutators,[54,55] as are strains that overproduce the enzyme.[56,57] The latter effect has been attributed to a reduced temporal window during which repair may occur. It is noteworthy in this context that a single hemi-modified GATC sequence is sufficient to direct *E. coli* mismatch repair and that this site may reside on either side of the mismatch.[58−60]

Although hemi-modification of GATC sites plays a major role in strand discrimination in vivo, a strand break will also suffice for this purpose.[61,62] In fact, as discussed below, the function of a hemi-methylated GATC site in *E. coli* mismatch repair is to provide a strand break, and it has been suggested that GATC modification is responsible for directing only a subset of mismatch repair events, the remainder attributed to strand discontinuities that occur naturally on daughter DNA strands during the course of DNA replication (a 3′-terminus on the leading strand, 3′- and 5′-termini on the lagging strand).[63] Strand discontinuities are also believed to be the natural strand discrimination signals in *Str. pneumoniae*.[64,65]

The *E. coli* methyl-directed system recognizes and repairs G−T, A−C, G−A, T−C, A−A, G−G, and T−T mismatches,[66−68] although some G−A and C−T mispairs are weak substrates, depending on sequence context.[67,69] The C−C mismatch is subject to little if any rectification. Insertion/deletion (ID) mismatches containing up to about four unpaired bases are also efficiently processed by the pathway.[70−73]

## 2.2. Biochemical Assays for Mismatch Repair

Molecular analysis of mismatch repair was made possible by the development of assays that permit mismatch rectification in vitro to be scored by biochemical or genetic methods. Both approaches have utilized circular heteroduplexes containing a mismatch and a strand discrimination signal. The biochemical assay relies on placement of a mismatch within overlapping recognition sites for two restriction endonucleases.[53,68,74,75] The mismatch renders the DNA resistant to cleavage by both endonucleases, but repair restores sensitivity to one or the other of the two endonucleases, depending on which strand is subject to rectification.

In the genetic method[76] a mismatch is placed within a $\beta$-galactosidase gene that resides within M13 viral DNA such that one strand contains a wild-type gene sequence while its complement contains a mutation that inactivates $\beta$-galactosidase function. After incubation with a cell-free fraction, DNA products are introduced into a mismatch-repair deficient *E. coli* strain, which is then plated on media containing 5-bromo-4-chloro-3-indolyl-$\beta$-D-galactopyranoside (X-gal), which yields a blue product upon hydrolysis by $\beta$-galactosidase. The fate of the mismatch is evaluated by scoring blue or white plaques versus plaques that display blue and white sectors. Plaques of a pure color arise as a consequence of repair, whereas sectored plaques reflect segregation of the two strands in the absence of correction.

## 2.3. The *Escherichia coli* Methyl-Directed Mismatch Repair Reaction

*E. coli* strains deficient in MutH, MutL, MutS, or DNA helicase II (also called UvrD) are deficient in methyl-directed mismatch repair.[49,53,66,77] Application of the in vitro restriction endonuclease assay described above permitted isolation of homogeneous preparations of MutH, MutL, MutS, and DNA helicase II.[62,78−80] Biochemical and genetic studies also implicated several additional activities in methyl-directed mismatch repair: exonuclease I (ExoI) exonuclease VII (ExoVII), RecJ exonuclease, exonuclease X (ExoX), single-stranded DNA binding protein (SSB), DNA polymerase III holoenzyme, and DNA ligase.[62,81−83]

Analysis of repair in *E. coli* extracts under conditions where repair DNA synthesis was blocked and study of reactions supported by purified proteins indicate that the overall repair reaction can be divided into several steps: mismatch-dependent incision of the unmethylated strand at a hemi-methylated GATC site; excision of that portion of the incised strand spanning the single-strand break and the mispair; repair of the ensuing gap by DNA synthesis and ligation (Figure 1). A key feature of this system is its bidirectional capability. The finding that a single hemi-methylated GATC sequence is sufficient to direct *E. coli* mismatch repair and that this site may reside on either side of the mispair suggested that the pathway could function in a bidirectional manner.[58−60] This was confirmed by use of electron microscopy and end-labeling methods to map excision tracts produced under conditions of repair synthesis block in both *E. coli* extracts and a reconstituted system comprised of purified forms of the proteins noted above.[84,85] The 6.4 kbp heteroduplexes used in these studies contained a G−T mismatch and a single hemi-methylated GATC site located about 1000 bp distant (as viewed along the shorter path linking the two sites on the circular DNA). Mismatch-provoked excision tracts were localized to the unmodified strand where they extended via the shorter path from the GATC site to terminate at a number of sites within a 100 nucleotide region beyond the mispair. Localization of excision tracts to the shorter path between the two DNA sites was observed irrespective of which strand of the helix harbored GATC modification, that is, whether the unmethylated GATC sequence was located 3′ or 5′ to the mismatch. These observations led to the suggestion that mismatch-provoked excision commences at the incised GATC site and proceeds toward the mispair.[85] It is pertinent to note in this context that while a hemi-modified GATC located 1000 bp from the mismatch can function efficiently in directing excision, the efficacy of the reaction decreases as the separation distance increases to 2000 bp.[58−60]

MutS is responsible for initiation of *E. coli* mismatch repair. This 95 kDa polypeptide, which exists as an equilibrium mixture of dimers and tetramers,[78,86] recognizes mismatched base pairs.[68,72,78,87] MutL, a 68 kDa polypeptide that is dimeric in solution, is recruited to the heteroduplex in a MutS- and ATP-dependent fashion.[80,88−92] The MutL•MutS•heteroduplex complex is believed to be a key intermediate in the initiation of mismatch repair, but as described below, its nature is not well understood.

Assembly of the MutL•MutS•heteroduplex ternary complex is sufficient to activate several downstream repair

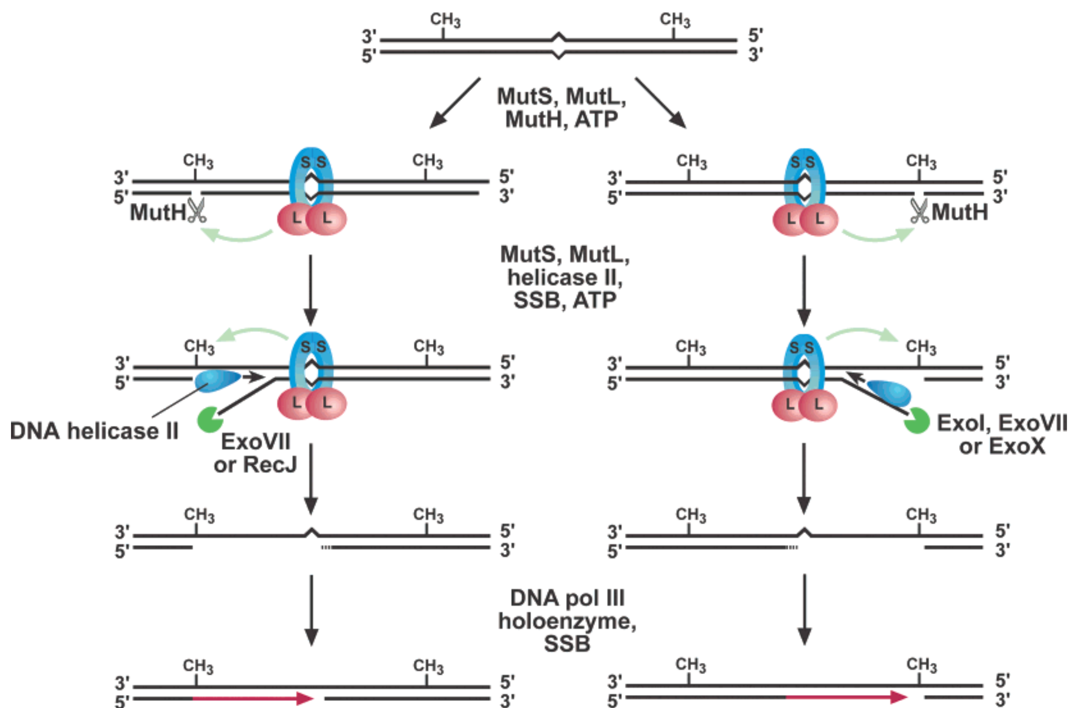


**Figure 1.** Mechanism of *E. coli* methyl-directed mismatch repair. Details of the reaction are described in the text. Although not shown, DNA ligase restores covalent continuity to the repaired strand after DNA polymerase III holoenzyme fills in the gap. Green arrows indicate MutS- and MutL-dependent signaling between the two DNA sites involved in the reaction.

activities. One of these is MutH, a 25 kDa latent endonuclease specific for unmodified GATC sequences. MutH is activated in a mismatch-, MutS-, MutL-, and ATP-dependent manner and incises the unmethylated strand of a hemimethylated GATC site 5′ to the G.[79,93] Activated MutH will also cleave both strands of an unmodified GATC site by a two-hit mechanism resulting in a double strand break. MutH incision can occur either 3′ or 5′ to the mispair on the unmodified strand, and the ensuing strand break serves as the actual signal that directs excision repair to the unmethylated strand (Figure 1). Thus, a preexisting single-strand break, which need not be within a GATC sequence, bypasses the requirements for both MutH and a hemi-modified GATC site in *E. coli* mismatch repair, an effect that has been documented both in vivo and in vitro.[61,62]

Formation of the MutS•MutL•heteroduplex complex is also sufficient to activate the methyl-directed excision system, which is comprised of DNA helicase II and several single-strand specific exonucleases. MutS and MutL activate unwinding by helicase II on nicked DNA in a mismatch-dependent manner.[94] Use of pre-steady-state methods demonstrated that helix unwinding in this system initiates at the strand break.[95] Although unwinding in this three-protein system occurs in both directions from the strand break, the reaction displays a fairly strong bias for unwinding toward the mismatch. Since this directional bias was observed without regard to placement of the nick 3′ or 5′ to the mispair, this finding led to the suggestion that MutS and MutL can coordinate recognition of the two DNA sites in a manner that establishes their relative orientation. This would permit orientation dependent loading of the helicase at the strand break so that unwinding proceeds toward the mismatch. That portion of the incised strand displaced by the helicase is subject to hydrolysis by an appropriate single-strand specific exonuclease (see below).

In the partial reaction systems described above, MutL plays a major role in the coupling of mismatch recognition by MutS to the activation of MutH or helicase II. This conclusion is based on the finding that under certain conditions MutL is sufficient to activate DNA helicase II on a substrate that lacks a mismatched base pair.[94,96,97] This effect has been attributed to physical interaction of the two proteins with MutL loading the unidirectional 3′ to 5′ helicase[98] onto the appropriate DNA strand so that unwinding proceeds toward the mismatch in a manner consistent with heteroduplex orientation. Under certain conditions, MutL can also activate the MutH endonuclease in a mismatch- and MutS-independent manner, an effect that has also been attributed to physical interaction of the two proteins.[99,100]

As noted above, mapping of excision tracts and the demonstration that MutS and MutL activate unwinding by DNA helicase II at a strand break have led to the conclusion that excision initiates at the site of MutH incision. This view is also consistent with the nature of exonuclease activities that have been implicated in methyl-directed repair based in vitro assay. Thus, when MutH incision occurs 5′ to the mismatch, excision depends on ExoVII or RecJ exonuclease,[82,85] both of which hydrolyze single-stranded DNA with 5′ to 3′ polarity.[101,102] When MutH cleavage occurs 3′ to the mispair, excision requires ExoI, ExoVII, or ExoX,[62,81−83,85] all of which support 3′ to 5′ hydrolysis of single-stranded DNA.[101,103,104] (ExoVII supports both 5′ to 3′ and 3′ to 5′ directionality.)

Genetic inactivation of both ExoVII and RecJ abolishes 5′-directed mismatch repair in *E. coli* extracts, whereas inactivation of ExoI, ExoVII, and ExoX is necessary to eliminate 3′-directed repair in vitro.[82,83] Similar heteroduplex orientation-dependent requirements for the exonucleases have been observed in reactions reconstituted using purified proteins.[82,83,85] ExoVII and RecJ thus provide redundant functions in 5′-directed excision, while ExoI, ExoVII, and ExoX provide redundancy in 3′-directed hydrolysis. Redundancy of ExoI, ExoVII, ExoX, and RecJ function in mismatch repair has also been documented in in vivo genetic

studies. Analysis of all possible single, double, and triple mutant strains failed to reveal a defect in mismatch repair as judged by mutability increase;[82,83,105] however, strains deficient in all four hydrolytic activities display a 7-fold increase in mutation rate, a value considerably less than the 50 to 100-fold increase in mutability conferred by helicase II or MutS defects.[83] While the limited increase in mutability associated with quadruple exonuclease deficiency might indicate that these enzymes play only a limited role in mismatch repair within the cell, this does not appear to be the case. Rather, the modest mutability of such strains is due to under recovery of mutations because bacterial chromosomes in which mismatches occur tend to be lost or destroyed.[81]

The single-stranded gap produced by the action of helicase II and a single-strand exonuclease is stabilized by SSB. In the absence of SSB, repair efficiency is reduced substantially.[62] DNA polymerase III holoenzyme is sufficient to support the repair synthesis step of methyl-directed correction in vitro, and extracts prepared from a *dnaZ*$^{ts}$ mutant have been shown to be defective in mismatch repair at the restrictive temperature.[62] In the final step of the reaction, helix integrity is restored by the action of DNA ligase.[62]

The *dnaZ* requirement indicates that the $\gamma$ complex of pol III holoenzyme is required for methyl-directed repair in vitro. The $\gamma$ complex functions as the loader that places the $\beta$ clamp onto the helix.[106] The $\beta$ clamp functions as a processivity factor for DNA polymerase III but has also recently been shown to interact physically with MutS.[107] As discussed below, recent work has demonstrated that PCNA (the eukaryotic homologue of the $\beta$ clamp) and the RFC clamp loader (homologue of the $\gamma$ complex) play important roles in regulation of mismatch-provoked excision in the human mismatch repair system. Unfortunately, potential effects of the $\gamma$ complex and the $\beta$ clamp on the excision step of bacterial mismatch repair have not been addressed, although such studies would appear warranted.

## *3. Mismatch Repair in Eukaryotes*

### 3.1. Mismatch Specificity and Strand Signals in Eukaryotic Mismatch Repair

As noted above, MutH incision at a hemi-methylated GATC site provides a DNA strand break that serves as the actual signal that directs *E. coli* mismatch repair. In fact, the initial demonstration of strand-directed mismatch repair in higher cells relied on the use of circular heteroduplex DNAs containing a strand-specific single-strand break.[75,76] Incubation of such DNAs with nuclear extracts derived from human or *Drosophila melanogaster* cells results in robust mismatch correction with the repair being directed to the incised strand. Such extracts support efficient nick-directed repair of G−T, A−C, A−A, G−A, G−G, T−T, C−T, and C−C mismatches, as well as small ID heterologies.[75,76,108−111]

Although strand discontinuities are sufficient to direct mismatch repair in vitro, the natural signals that direct the eukaryotic reaction remain uncertain. Early studies[112−114] suggested that cytosine methylation might be involved in strand discrimination in mammalian cells in a manner analogous to the role of adenine methylation in *E. coli*. However, more recent work has seemingly ruled out this possibility.[115,116] On the other hand, several groups have found that mouse cells deficient in the DNA cytosine methyltransferase Dnmt1 display instability of mono- and dinucleotide repeat sequences,[117,118] a phenotype characteristic of mismatch repair-deficient cells.[41,119,120] Although a role for Dnmt1 in mammalian mismatch repair has been inferred on these grounds, defective or aberrant repair in Dnmt1-deficient cells has not been directly demonstrated.

Other possible mechanisms for strand discrimination in eukaryotic cells have also been considered. Strand discontinuities occurring naturally as intermediates during the course of DNA replication may provide strand signal functions in bacterial mismatch repair and could function in a similar manner in eukaryotic systems.[27] Wagner and Meselson postulated that “special relation to the replication complex” could effect strand discrimination in mismatch repair.[48] As detailed below, the PCNA replication clamp interacts with several eukaryotic mismatch repair activities, and it has been suggested that PCNA might provide a physical link between repair and replication that would allow DNA termini at the fork to function as strand signals.[121] Yet another possibility is that noncovalent signals in the form of proteins that segregate with the individual strands during replication could conceivably provide a mechanism for discrimination of parental and nascent strands.[27]

### 3.2. Eukaryotic MutS and MutL Homologues

Genes encoding homologues of bacterial MutS and MutL have been identified in a variety of eukaryotes including yeast, plants, insects, nematodes, and mammals,[26−30,122−124] although no eukaryotic homologue of MutH has been identified. The several eukaryotic homologues of MutS have been designated MSH1−MSH6. MSH1, which has not been identified in mammalian cells, is required for mitochondrial DNA stability in *Saccharomyces cerevisiae*.[12,125,126] Eukaryotic MSH2, MSH3, and MSH6 gene products have been implicated in mitotic genetic stability where they participate in repair of base−base mismatches and ID heterologies;[12,14,27,110,127−141] however, MSH2 has also been implicated in meiotic gene conversion.[12] Function of the MutS homologues MSH4 and MSH5 is apparently restricted to meiosis where they play important roles in crossing over in both yeast and mammals.[142−146]

Genes that encode MutL homologues have also been identified in eukaryotes. MLH1 and PMS2 (mammalian PMS2 corresponds to PMS1 in yeast, plants, and nematodes) have been the most extensively characterized. Both have been implicated in mitotic mutation avoidance[110,128,147−154] and in meiotic recombination.[11,155−157] MLH3, which has been identified in yeasts and mammals[158,159] plays an important role in meiotic crossing-over[157,160,161] but also functions in mitotic genetic stabilization in yeast by preventing frameshift mutations.[158,162] Mitotic functions of MLH3 in mammalian cells have been the subject of controversy.[159,163−166] Other MutL homologues have also been identified: MLH2 in yeast[157,162] and PMS1 in humans.[167] The former protein may provide a meiotic function.[157]

Available evidence indicates that eukaryotic MutS and MutL homologues function as heterodimers, which allows for a modular system for recognition and processing of different types of DNA lesions. In both human and yeast, MSH2 forms a heterodimer with MSH6 (MutS$\alpha$) or MSH3 (MutS$\beta$).[130,131,135,136,138,140,141,168−170] In human cells, MSH2 partitions between MSH6 and MSH3 such that about 85% of the MSH2 is found in the MSH2·MSH6 MutS$\alpha$ complex.[140,141] Human MutS$\alpha$ supports repair of all eight base−base mismatches including C−C, as well as ID mispairs containing up to about 10 unpaired nucleotides, whereas

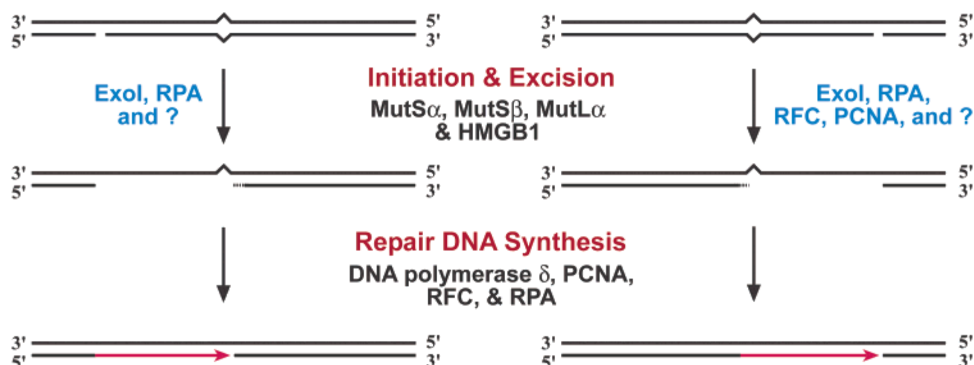


**Figure 2.** Human bidirectional mismatch repair in vitro. Human mismatch repair in vitro can be directed by a strand break located either 3′ or 5′ to the mismatch. Activities that have been implicated in several steps of the reaction are shown. Question marks indicate that unidentified activities may also play significant roles in the reaction.

MutS*β* supports correction of ID mismatches containing two to about 10 nucleotides but is only weakly active on single nucleotide ID mispairs.[138,141] This specificity is consistent with the finding that mononucleotide (but not dinucleotide) repeat instability is diagnostic for MSH6-deficient tumor cells.[120,171]

As judged by mutation spectra and in vitro heteroduplex binding assay, the specificities of yeast MutSα and MutS*β* are similar to their human counterparts. Yeast MutSα recognizes base−base mismatches (C−C is a weak substrate), as well as ID mispairs of up to about 10 unpaired nucleotides.[147,169,170,172,173] Yeast MutS*β* supports repair of ID mismatches of one to about 10 unpaired nucleotides.[134,136,173−175]

Although not as well studied as their MutS counterparts, eukaryotic MutL homologues also appear to function as heterodimers, MLH1 serving as a common subunit. The best characterized of these has been MutLα, which has been isolated from both human (MLH1·PMS2 heterodimer) and yeast (MLH1·PMS1 complex)[153,176−178] and is capable of supporting repair initiated by MutSα or MutS*β*.[130,176,179] Formation of MLH1·MLH2 and MLH1·MLH3 complexes has been inferred on genetic grounds in *S. cerevisiae*, the latter complex cooperating with MutS*β* to prevent frameshift mutations.[157,158,162] A human MutL*β* complex of MLH1 and PMS1 has also been isolated, but its molecular activities have not been ascertained.[180,181]

## 3.3. Mismatch Repair in Cell-Free Extracts

Much of the information on the nature of eukaryotic strand-directed mismatch repair has derived from study of the nick-directed reaction in human cell-free extracts (Figure 2). As in the bacterial reaction, the nick that directs repair can be located 3′ or 5′ to the mismatch.[108,182] The rate of nicked-directed correction diminishes with an increase in nick−mismatch separation distance from 125 to 1000 bp, although repair is readily demonstrable at the larger distance.[108] As discussed above, electron microscopy has been employed to directly visualize excision tracts produced by the bacterial methyl-directed system when repair DNA synthesis is blocked. These experiments demonstrated the presence of a single-stranded gap spanning the distance between the mismatch and the GATC site that directs repair. Although excision tracts produced in extracts of human cells have not been visualized in this manner, several lines of evidence suggest a similar mode of excision. Radiolabeled nucleotide incorporation into nicked heteroduplexes occurs preferentially in the region spanning the nick and the mispair.[75,76] Furthermore, end-labeling studies have demonstrated that nick-directed, mismatch-provoked excision (under conditions of repair synthesis block) leads to production of a new set of DNA termini localized to a region 90−170 nucleotides beyond the mispair.[108] A gap extending from the nick to this set of sites has been inferred on the basis of conversion of this region to a restriction endonuclease-resistant form and by virtue of its ability to serve as a hybridization acceptor for complementary oligonucleotides.[108,182]

An alternate mode of excision has been suggested on the basis of analysis of radiolabeled nucleotide incorporation into nicked heteroduplexes in *Xenopus* egg extracts. Fine structure restriction analysis of repair products demonstrated a significantly higher specific radioactivity in the vicinity of the mismatch than near the strand break.[183] On the basis of this analysis, Radman and colleagues have suggested an alternate mode of excision wherein the nick that directs repair serves only as a strand signal, rather than an initiation site for excision.[183] In this model a mismatch-stimulated endonuclease is postulated to introduce a strand-specific nick in the vicinity of the mismatch. This nick serves as the site for initiation of excision, which is restricted to the immediate vicinity of the mispair.[183] It is not clear whether these different conclusions concerning the modes of excision in human and *Xenopus* extracts are due to biological or experimental differences in these two systems. However, it is pertinent to note that a similar radiolabel incorporation study in HeLa cell extracts demonstrated that the highest label enrichment occurred in the vicinity of the strand break that directs repair.[76] On the other hand, much of the fine structure mapping of excision tracts in the human system has relied on analysis of single-stranded gaps produced under conditions of repair DNA synthesis block, a condition that could conceivably perturb the experimental outcome.[108,182]

Analysis of nick-directed repair in crude and partially fractionated extracts has implicated a number of activities in the human reaction. MutSα, MutS*β*, and MutLα were initially identified on the basis of repair defects in extracts of hypermutable tumor cell lines resulting from deficiency of one or more of these activities.[130,131,135,138,140,141,153,184]

As discussed above, DNA helicase II and multiple exonucleases participate in the excision step of bacterial mismatch repair. By contrast, there is no compelling evidence for helicase involvement in eukaryotic mismatch repair,[185−187] and only one hydrolytic activity has been convincingly implicated in the reaction. Exonuclease I (ExoI) is a member of the Rad2 family that hydrolyzes duplex DNA with 5′ to 3′ polarity but also displays 5′ flap endonuclease activity.[188−193]

Initial evidence for ExoI involvement in mismatch repair was obtained in yeast. Yeast *exoI* mutations confer a mutator phenotype, and yeast ExoI has been shown to interact with yeast MSH2.[189,190,194] Similarly, the human ExoI homologue has been found to interact with human MSH2, MLH1, and MSH3.[195−197] Direct evidence for participation of ExoI in nick-directed mismatch repair was provided by experiments in which human nuclear extracts were depleted of ExoI.[198] Surprisingly, depletion of the activity attenuated not only 5′-directed excision and repair but 3′-directed reactions as well, although both could be restored by supplementation with homogeneous human ExoI.[198] Since a similar requirement for ExoI in 5′- and 3′-directed repair has been observed in $ExoI^{-/-}$ mouse cells,[199] the 5′ to 3′ exonuclease is evidently required for both excision directionalities supported by the system.

Involvement of several other hydrolytic activities in eukaryotic mismatch repair has also been suggested, but evidence in these cases is less compelling. Analysis of dinucleotide repeat instability in *S. cerevisiae* led to the suggestion that the RAD27 exonuclease may be involved in mismatch repair,[200] but subsequent studies demonstrated that this activity has little if any role in mismatch correction.[201,202] The editing exonuclease functions of DNA polymerases $\delta$ and $\epsilon$ have also been postulated to provide hydrolytic functions in mismatch repair;[203,204] however, this suggestion has also been questioned.[205]

Mismatch repair in nuclear extracts is insensitive to DNA polymerase $\beta$ inhibitors but is abolished by aphidicolin, an inhibitor of the eukaryotic replicative polymerases α, $\delta$, and $\epsilon$.[75,76,108] Repair is also reduced by low concentrations of butylphenyl-dGTP, a nucleotide that inhibits all three DNA polymerases but preferentially inhibits polymerase α at the concentrations used.[76] The nature of the repair synthesis step of mismatch correction was clarified by development of a depleted extract system that sustains mismatch-provoked excision but fails to support the complete repair reaction.[206] A HeLa activity that restored repair to the depleted extract was isolated and shown to be identical to DNA polymerase $\delta$ with highly purified fractions devoid of detectable α or $\epsilon$. Additional evidence for polymerase $\delta$ involvement in mismatch repair has been provided in yeast where genetic studies have shown that mutations in *POL32*, which encodes a noncatalytic subunit of polymerase $\delta$, potentiate the mutability of ExoI-deficient strains.[194] Thus, DNA polymerase $\delta$ is required for eukaryotic mismatch correction, but supporting roles for polymerases α and $\epsilon$ have not been ruled out.

Analysis of nick-directed mismatch repair in nuclear extracts of human cells has also implicated several DNA binding proteins in the reaction. Involvement of the human single-stranded DNA binding protein RPA was established by immunological methods, and the protein has been shown to stabilize excision intermediates and to facilitate repair DNA synthesis in crude fractions.[207,208] Use of a depleted extract approach similar to those described above has also suggested involvement of HMGB1, a non-histone chromatin protein. This 30 kDa protein, which binds to certain types of DNA damage,[209] interacts with MutSα and may play an important role in early steps of the reaction prior to excision.[210]

PCNA, the eukaryotic replication sliding clamp, also plays several important roles in mismatch repair.[121,211] Given that PCNA is an important cofactor for DNA synthesis by polymerase $\delta$,[212] its involvement in the repair synthesis step of mismatch correction is not surprising.[213] However, depletion of PCNA from human cell extracts by p21, which binds tightly to PCNA and effectively sequesters the protein,[214,215] abolishes 3′-directed, mismatch-provoked excision and inhibits 5′-directed excision to a limiting level of about 50%.[121,216,217] Additional support for PCNA involvement in mismatch repair has been provided in the yeast system with the identification of mutant alleles within the PCNA structural gene that display elevated mutability.[121,211,218−220]

Much of the work on the nature of PCNA involvement in early steps of mismatch repair has focused on interaction of the protein with MutSα and MutS$\beta$. Although a robust interaction between PCNA and MutLα has not been demonstrated,[221,222] PCNA interacts strongly with MutSα and MutS$\beta$, both of which harbor a PCNA recognition motif located near the N-terminus of the MSH6 or MSH3 subunit, respectively.[211,221,223,224] Interestingly, mutations within yeast structural genes for MSH3 or MSH6 that abolish the MutSα−PCNA and MutS$\beta$−PCNA interactions in vitro display only a modest mutability increase in vivo,[220,223,224] suggesting that this interaction plays a significant but nonessential role in mismatch repair.

Similar results have been obtained in the human system on the basis of analysis of MutSα−PCNA interaction. The PCNA binding motif of human MutSα resides within the N-terminal 12 amino acids of the MSH6 subunit. Unlike native MutSα, a variant lacking the 77 MSH6 N-terminal amino acids fails to colocalize with PCNA to replication foci in vivo and is defective in its ability to restore nick-directed repair to extracts derived from an MSH6-deficient cell line.[221] By contrast, two other human MutSα variants (lacking the N-terminal 12 or 341 amino acid residues of MSH6) that interact poorly with PCNA have been found to support near normal levels of mismatch repair upon supplementation of MSH6-deficient extracts (R. Iyer, T. Pohlhaus, S. Chen, and P. Modrich, unpublished). Although the latter findings are consistent with the yeast studies mentioned above, the differing results obtained with the different N-terminal MSH6 truncations have not been resolved.

## 3.4. Reconstituted Eukaryotic Mismatch Repair Reactions

The functions of ExoI and PCNA in eukaryotic mismatch repair have been further clarified by establishment of several reconstituted systems that support mismatch-provoked excision by purified human proteins. The simplest of these (Figure 3), which is comprised of MutSα, MutLα, ExoI, and RPA, supports a mismatch-provoked excision reaction that occurs exclusively with 5′ to 3′ directionality,[198,216] consistent with the 5′ to 3′ polarity of ExoI hydrolysis in the absence of other proteins.[191−193] Although MutLα is not essential for excision in this system, it does enhance the mismatch dependence of the reaction.

In the absence of RPA, MutSα stimulates ExoI hydrolysis of nicked DNA in a mismatch- and ATP-dependent manner.[216] Under these conditions, MutSα renders ExoI highly processive, an effect attributed to formation of a complex between the two proteins on heteroduplex DNA. RPA modulates behavior of this complex, reducing processivity from ∼2000 to ∼250 nucleotides. This leads to termination of excision upon mismatch removal as a consequence of two effects. An RPA-filled gap is an extremely poor substrate for ExoI, but MutSα promotes ExoI initiation at such sites

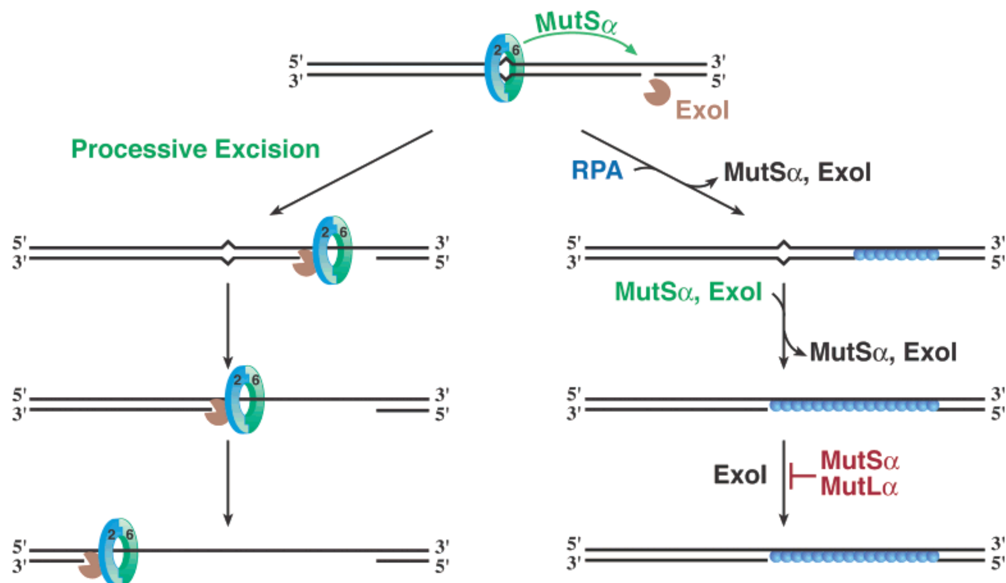


**Figure 3.** RPA regulation of mismatch-provoked 5′ to 3′ excision. As described in the text, MutSα confers a high degree of processivity on ExoI presumably via formation of a molecular complex between the two proteins (left). RPA reduces the processivity of this complex to about 250 nucleotides (right). An RPA-filled gap is an extremely poor substrate for ExoI reloading, but MutSα can promote reloading if the DNA contains a mismatched base pair. Hence, in the presence of RPA, excision is dramatically attenuated upon mismatch removal because MutSα can no longer assist in this manner. This effect is potentiated by MutSα and MutLα, which act together to suppress ExoI activity on DNA that lacks a mismatch, leading to effective termination of excision.

provided that the DNA contains a mismatched base pair. Hence, excision is dramatically attenuated upon mismatch removal because MutSα can no longer assist in this manner. This effect is potentiated by MutSα and MutLα, which act together to suppress ExoI hydrolysis on DNA that lacks a mismatched base pair, leading to effective termination of excision.[216] Interestingly, this simple mechanism provides a potential explanation for one of the most puzzling questions in mismatch repair. In both the bacterial and human systems, excision terminates at a number of sites centered about 100 nucleotides beyond the original location of the mispair. The mechanism of this four protein purified system suggests that termination in this manner may be a simple consequence of the distance separating the nick and the mispair coupled with the degree of processivity of the hydrolytic system, that is, the number of times the excision system must be reloaded to effect mismatch removal.

The 5′ to 3′ directionality of this four protein system can be regarded as a default polarity because hydrolysis always proceeds 5′ to 3′ from the strand break without regard to nick location 5′ or 3′ to the mismatch. Thus, when the nick that directs excision is 5′ to the mispair, hydrolysis proceeds 5′ to 3′ toward the mismatch, terminating upon mismatch removal. However, the four protein system also supports mismatch-provoked excision on a 3′-heteroduplex, and in this case, hydrolysis also proceeds with 5′ to 3′ polarity, which is incorrect directionality for mismatch removal.[198,222] This observation led to the finding that supplementation of MutSα, MutLα, ExoI, and RPA with PCNA and RFC (the enzyme that loads the PCNA clamp onto the helix)[225] yields a system that supports bidirectional excision.[222] When the nick that directs the reaction is located 5′ to the mismatch, hydrolysis in this six-component system proceeds 5′ to 3′ as it does in the default pathway. However, when the nick is located 3′ to the mispair, 5′ to 3′ hydrolysis by ExoI is suppressed, and excision proceeds with apparent 3′ to 5′ polarity, resulting in mismatch removal (Figure 4). The apparent 3′ to 5′ polarity of excision in this system assumes that the hydrolytic events leading to mismatch removal

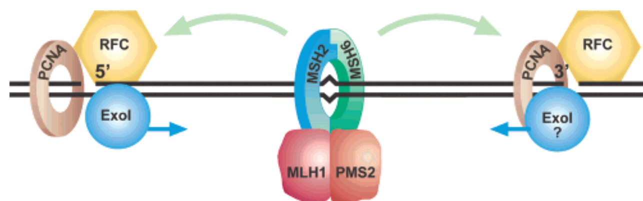


**Figure 4.** A purified human system that supports bidirectional excision. A six-component system comprised of MutSα, MutLα, ExoI, RPA, RFC, and PCNA supports mismatch-provoked excision directed by a strand break located either 3′ or 5′ to the mismatch. Both 3′- and 5′-directed excision reactions depend on integrity of the ExoI active site. The simplest explanation for this finding is that ExoI mediates both 3′- and 5′-directed excision; this point has not been established. Green arrows indicate that the reaction is dependent on signaling between the two DNA sites. Reprinted with permission from ref 222. Copyright 2004 Elsevier, Inc.

initiate at the strand break that directs the reaction, but this point has not been established.[222] Nevertheless, this six-component system displays the key element of bidirectional excision that has been observed in nuclear extracts, namely, activation of differential excision events in response to 3′- or 5′-heteroduplex orientation.

3′-Directed excision in the six-component system requires MutSα, MutLα, ExoI, PCNA, RFC, and ATP with RPA stimulating the reaction. The ExoI requirement for both 5′- and 3′-directed excision in the reconstituted bidirectional system is similar to the bidirectional requirement for the protein in nuclear extracts.[198,199] Although the role of the 5′ to 3′ exonuclease in 3′-directed excision is not clear, analysis of an active site mutant has demonstrated a requirement for the ExoI catalytic center in both 5′- and 3′-directed reactions.[222] Several possible explanations could account for this finding. ExoI could play a structural role in the assembly of a multiprotein repair complex required for activation of 3′-directed excision by an as yet unidentified activity associated with another repair protein;[194] however this possibility is somewhat difficult to reconcile with the ExoI active site requirement in 3′-directed excision. It has also been suggested

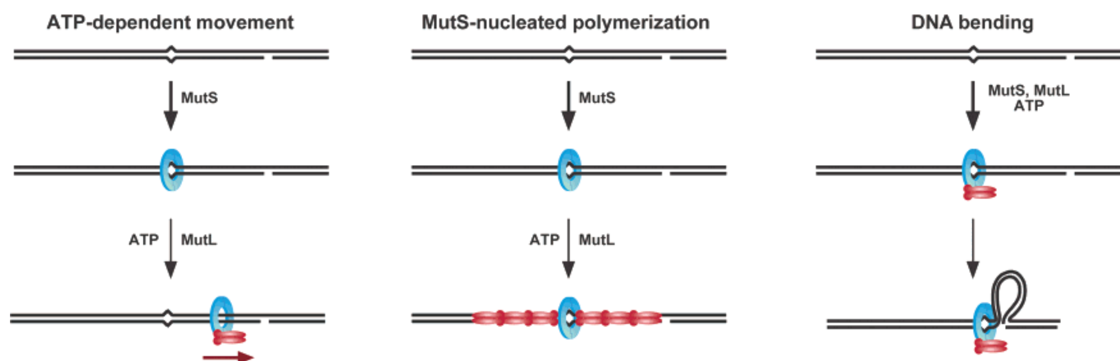


**Figure 5.** Mechanisms for signaling between the mismatch and the strand signal. The models depicting ATP-dependent movement, MutS-nucleated polymerization, and DNA bending are described in the text.

that ExoI may harbor a cryptic 3′ to 5′ hydrolytic activity that is activated on a 3′-heteroduplex by other components of the repair system[222] with this activity presumably sharing active site residues with the 5′ to 3′ hydrolytic function. A third possibility is that excision with apparent 3′ to 5′ directionality is dependent in some way on 5′ to 3′ hydrolysis by ExoI.

Use of a mutant form of the protein and domain specific antibodies has indicated that RFC provides at least two functions in the reconstituted bidirectional excision system.[222] The amino terminal ligase homology domain of the large RFC subunit, which is not required for PCNA loading onto the helix,[226,227] is necessary for suppression of 5′ to 3′ hydrolysis from a 3′-strand break but is not required for activation of 3′-directed excision. Domain B of the large RFC subunit, which functions in PCNA loading,[228] is not necessary for suppression of 5′ to 3′ hydrolysis from a 3′-nick but is required for activation of 3′-directed excision. Based on the latter finding, it has been inferred that the loaded form of PCNA is required to activate 3′-directed hydrolysis.[222] Although PCNA has been suggested to function as a strand signal during mismatch repair,[121,229] these results suggest that RFC and PCNA function to regulate directionality of excision.[222] The manner in which PCNA is loaded onto the helix provides a simple mechanism by which this might occur. PCNA is loaded at strand discontinuities in an orientation-dependent manner, that is, different faces of the PCNA clamp would be oriented toward the mismatch in 3′ and 5′ heteroduplexes.[212] Inasmuch as PCNA and ExoI interact, the PCNA orientation at the strand break could be exploited to control directionality of excision (Figure 4).[222]

As mentioned above, HMGB1 has been implicated in early steps of nick-directed mismatch in human cell extracts. However, the reconstituted bidirectional system does not display an obvious requirement for this protein. The differential requirement for HMGB1 in the two systems could be the consequence of the presence of other DNA binding activities in nuclear extract.[222] Such proteins could restrict access of repair activities to the mismatch or strand discontinuity in heteroduplex DNA, and HMGB1 may function to reverse this type of effect in the extract system. The absence of such activities in the purified system would obviate the requirement for the protein.

As discussed above, DNA polymerase $\delta$ has been implicated in both human and yeast mismatch repair systems. In fact, supplementation of the reconstituted, six component bidirectional excision system with DNA polymerase $\delta$ yields a system that supports mismatch correction directed by either a 3′- or 5′-strand break.[230] While availability of these reconstituted reactions should facilitate additional work on the mechanism of eukaryotic mismatch repair, it is important to note that they should be regarded as minimal systems for several reasons. Analysis of ExoI-depleted nuclear extracts and the phenotype of ExoI-deficient mouse cells has indicated that the probable existence of other excision activities that may function in a redundant manner with respect to ExoI.[198,199] Furthermore, the mismatch dependence of reconstituted 5′-directed excision is not as dramatic as that observed in nuclear extracts[216,222] indicating that one or more specificity factors may be lacking. These activities, as well as the ligase responsible for termination of repair, remain to be identified.

## 4. Coupling of Mismatch Recognition and Strand Discrimination

Distinct excision responses are elicited in the bacterial and human repair systems depending on whether the strand break that directs the reaction is located 3′ or 5′ to the mismatch. The repair systems must therefore establish the relative orientation of these two sites on the heteroduplex, which can be separated by 1000 bp or more. Three types of model have been proposed to explain the mode of interaction of the two DNA sites (Figure 5). One model posits ATP-dependent movement of a MutS homolog, as well as the corresponding MutS•MutL complex, from the mismatch to the strand signal along the helix contour.[91,231−234] A second model postulates that the mismatch•MutS homologue complex serves as a nucleation site for polymerization of a second protein along the helix, with the obvious candidate being the corresponding MutL homolog.[235,236] The common theme of these two models is signal transduction along the helix contour, which can in principle account for the capability of the repair system to respond to heteroduplex orientation, that is, strand signal placement 3′ or 5′ to the mismatch. A third transactivation model stipulates that MutS and MutL homologues remain bound to the mismatch with activation of downstream activities at the strand signal mediated by a DNA bending mechanism.[90,237,238] Despite extensive work in a number of laboratories, the molecular mechanism responsible for signaling in mismatch repair has not been established. The sections immediately below will highlight work that bears on this problem.

### 4.1. ATPase Activities of MutS and MutL Homologues

Mismatch-, MutS- and MutL-dependent activation of the bacterial MutH GATC endonuclease requires ATP and is

inhibited by ATPγS,[93] implicating ATP hydrolysis in the interaction of the two DNA sites involved in the *E. coli* methyl-directed reaction. Accordingly, much of the work on signaling in mismatch repair has addressed modulatory effects of adenine nucleotides on the interaction of MutS and MutL homologues with DNA. Requirements for ATP hydrolysis (as opposed to nucleotide binding) have been inferred in such studies on the basis of comparative analysis of ATP effects with those obtained using nonhydrolyzable analogues and by use of mutant proteins defective in ATP binding or hydrolysis or both.

MutS homologues are members of the ABC (adenine nucleotide binding cassette) transporter family of ATPases[239] and hydrolyze ATP with modest turnover numbers.[91,93,240−250] In the absence of DNA, reported turnover numbers for *E. coli* MutS are 2−26 $min^{-1}$ per dimer,[91,237,245,251,252] 80−240 $min^{-1}$ for the *Thermus aquaticus* MutS dimer (at 70−80 °C),[247,250] 7−14 $min^{-1}$ for the yeast MutSα,[169,253] and 0.2−1 $min^{-1}$ for human MutSα.[243,244,246,254] These values were obtained under a variety of experimental conditions, and some of the variability, especially those values on the higher side, may be due to presence of ATPase contaminants in the preparations used.

The general consensus is that the ATP hydrolytic activity of MutS homologues is activated by DNA,[91,243−246,248−250,255] although the degree of activation depends on the experimental system. Thus, DNA activates the *E. coli* MutS ATPase about 4-fold,[91,245] the human MutSα ATPase 10−20-fold,[243,244,246] and the yeast MutSα ATPase about 4-fold.[248] At physiological ionic strength, heteroduplex DNA is a significantly better activator than homoduplex DNA in each of the three systems,[91,244,246,248,256] but *T. aquaticus* MutS appears to be an exception to this rule.[250] MutS homologue mutants defective in ATPase function typically retain mismatch recognition activity and can display a dominant negative mutator phenotype in vivo.[92,240,242,248,252,254,257−259]

MutL homologues belong to a structurally distinct family of ATP binding proteins, typified by HSP90, type II DNA topoisomerases, and histidine kinases.[260,261] Like MutS homologues, bacterial MutL and eukaryotic MutLα are weak ATPases.[89,178,262,263] The *E. coli* MutL ATPase is dramatically activated by single-stranded DNA, but less so by duplex DNA.[89,262] By contrast, the ATPase activity of human MutLα does not respond to DNA,[178,263] although the N-terminal ATPase domain of the PMS2 subunit binds DNA with a preference for duplex molecules.[263] Integrity of the MutL homologue ATP center is required for function in mismatch repair[89,178,259,264,265] and, in the case of the *E. coli* protein, is necessary for both MutH activation and mismatch-provoked excision.[89]

## 4.2. Structural Features of Bacterial MutS and MutL

Since structures of bacterial MutS and MutL have been the subject of several recent reviews,[31,266−269] only salient structural features of the proteins will be summarized here. Structures have been solved for a near full length, C-terminal truncated form of *T. aquaticus* MutS complexed with an unpaired T heteroduplex[270] and for a similar *E. coli* MutS variant complexed with G−T, C−A, A−A, G−G, and unpaired T heteroduplexes.[271,272] These structures are strikingly similar. In all, the truncated MutS forms a dimeric, clamp-like structure about the heteroduplex. The shape of the individual subunits has been likened to a "comma",[270] and the structure of the dimer is similar to the Greek letter $\theta$,[267] with two large adjacent channels. Heteroduplex DNA is threaded through the larger of these.[268,270,271] The functional significance of the empty channel is not known, but its size and charge suggest that it might also be able to accommodate a DNA segment.[267] Interactions between the highly conserved ATPase domains of the two subunits, which are located at the distal end of the dimer relative to the heteroduplex channel, provide much of the stabilization energy for dimer formation.[270,271]

Heteroduplex binding is mediated by an N-terminal clamp domain, which is comprised of long α-helical arms and a highly conserved mismatch recognition domain. The former serves to clamp the DNA within the dimer, and the latter provides heteroduplex recognition contacts.[270−272] Although comprised of two identical polypeptide chains, the heteroduplex-bound dimer is structurally asymmetric with mismatch recognition contacts provided by only one subunit. In both the *T. aquaticus* and *E. coli* structures, the unpaired or mispaired base(s) remain intrahelical and the phenylalanine of the Phe-X-Glu mismatch recognition motif within one subunit intercalates into the helix via the minor groove to stack with a mispaired base.[270−272] The recognition motif glutamic acid hydrogen bonds to the same base (to N7 if the base is a purine and to N3 if it is a pyrimidine). These interactions result in a heteroduplex kink of about 60°,[270,271] an observation that has led to the suggestion that MutS homologues may exploit helix deformability conferred by a mispair during the course of mismatch recognition.[267,270,271] However, recent atomic force microscopy studies have demonstrated that while virtually all MutS·homoduplex complexes are kinked with a bend angle of 40°−60°, bend angles observed with MutS·heteroduplex complexes are bimodal with a significant fraction of the complexes largely unbent.[273] This has led to the proposal that kinked MutS·DNA complexes, which also form in regions of perfectly paired helix, represent an intermediate on the path to an ultimate unbent mismatch recognition complex.

The truncated forms of MutS used in crystallographic studies may be significantly compromised with respect to biological activity. *E. coli* MutS800, which lacks the C-terminal 53 amino acids, is unable to form tetramers, exhibits a reduced ability to support MutH activation, and is defective in its ability to suppress homeologous recombination or mediate a normal cisplatin response (the latter MutS functions are considered below).[86,274] Estimated dissociation constants for the tetramer to dimer transition of native MutS range from $10^{-7}$ M (0.1 M KCl, 5 mM $MgCl_2$, 4 °C)[86] to $10^{-6}$ M (0.25 M NaCl, 10 mM $MgCl_2$, 20 °C),[275] although the latter value is based on a single concentration at one rotor speed. These values should be compared to an estimated intracellular MutS concentration of about 0.5 $\mu$M.[276] It is also noteworthy that the MutS tetramer is not simply a dimer of dimers; rather, the tetramer can bind only one heteroduplex molecule.[86]

Although a structure is not available for full length MutL, structures have been determined for a conserved N-terminal fragment of the *E. coli* protein that includes the ATPase domain (residues 1−349) and for a C-terminal dimerization domain (residues 432−615).[99,262,277] The C-terminal domain of MutL crystallizes as a V-shaped dimer. In the absence of nucleotide the N-terminal ATPase domain is monomeric and elbow shaped, and about 60 residues are present in disordered loops.[99,262] However, in the presence of nonhydrolyzable

AMPPNP, these loops become ordered and mediate association of two ATPase monomers to form a saddle-shaped dimer, which is postulated to disassemble upon nucleotide hydrolysis.[99,262] The C-terminal dimerization domain has been modeled to dock on the posterior side of the ATPase dimer, thus creating a central channel, the dimensions of which are determined by the 83 amino acid linker of unknown structure that joins the N-terminal ATPase with the C-terminal dimerization domain.[277] DNA is postulated to thread through this channel in the ATP-bound, closed form of the dimer. DNA access to the channel would be determined by nucleotide occupancy of the ATPase domain, with entry or exit allowed upon ATP hydrolysis, which attenuates interaction between N-terminal ATPase domains to produce the open form of the dimer.

Yeast two-hybrid studies have suggested that MutH interacts with the C-terminal portion of MutL.[100] However, cross-linking experiments and structure-based modeling studies indicate that MutH interacts with the N-terminal portion of MutL and that this interaction depends on ATP-mediated dimerization of the MutL ATPase domain.[266,278,279] On the other hand, both N- and C-terminal domains of MutL are thought to interact with DNA helicase II.[277]

## 4.3. Interaction of DNA Binding and ATPase Centers in MutS Homologues

Modulatory effects of DNA on ATP hydrolysis and nucleotide effects on MutS homologue–DNA interaction indicate that the DNA binding site and ATPase centers interact strongly. Pre-steady-state chemical quench studies with *E. coli* MutS, *T. aquaticus* MutS, and yeast MutSα have demonstrated an initial burst of ADP formation, implying that hydrolytic chemistry is fast relative to turnover in the absence of DNA.[245,249,250] Occurrence of the ADP burst is not altered by homoduplex DNA, but it is abolished in the presence of heteroduplex DNA. This implies that mismatch recognition increases the lifetime of MutS-bound ATP and that in the presence of heteroduplex DNA the rate-limiting step for turnover occurs at or prior to hydrolysis. Thus, occupancy of the DNA binding site modulates activity of the ATP hydrolytic center.

Conversely, nucleotide binding site occupancy regulates MutS homologue interaction with DNA, and as might be expected from such observations, MutS undergoes a conformational change upon ATP binding.[252,275,280,281] However, modulatory effects of adenine nucleotides on MutS homologue–DNA interaction are not well understood. To some extent, this may reflect the number of permissible occupancy states available to the two ATPase centers. If the minimal functional unit of a MutS homologue is assumed to be an asymmetric dimer, then the two nucleotide-binding sites can in principle be filled in nine different ways. Analysis of nucleotide binding by *E. coli* MutS, *T. aquaticus* MutS, yeast MutSα, and human MutSα has demonstrated that in the absence of DNA, each has one high-affinity site for ADP and one for a nonhydrolyzable ATP analogue per dimer equivalent. Furthermore, the two classes of sites can be simultaneously occupied by ADP and an ATP analogue, suggesting that the ADP•S•ATP (S corresponds to MutS or MutSα) species may be highly populated in solution.[249,250,282,283] Indeed, examination of nucleotide occupancy during the course of a single ATP hydrolytic turnover has demonstrated that the mixed occupancy species have significant lifetimes.[250,283] Despite the intrinsic specificities of the two sites, high nucleotide concentrations support formation of an ATPγS•S•ATPγS complex with *T. aquaticus* MutS and yeast MutSα, and an ADP•S•ADP complex has been demonstrated for the former protein.[249,250] Neither of these complexes has been observed with human MutSα, and the latter species has not been detected for yeast MutSα,[249,283] but the failure to detect such complexes in these instances could be due to lifetimes too short for their collection on the nitrocellulose membranes used for their detection. In fact, the ATP•S•ATP and ADP•S•ADP complexes have been postulated to play significant roles in MutS homologue function.[250]

Initial attempts to evaluate ATP effects on MutS–DNA interaction were based on visualization of complexes of the *E. coli* protein with 6.4 kbp heteroduplex and homoduplex DNAs by electron microscopy. These experiments demonstrated the mismatch- and ATP-dependent formation of α-shaped DNA loop structures up to several kilo-base pairs in size, which were stabilized by MutS bound at the base.[231] Although the bound protein was initially interpreted to be the dimer, more recent work has led to the conclusion that it was probably the tetramer.[86] Loop size was found to increase with time, and in the majority of the molecules, the mismatch was present in the loop. Nonhydrolyzable ATP analogues failed to support large loop formation, and ongoing loop growth was suppressed upon their addition to ATP-containing reactions.[231] These effects were attributed to a translocation mechanism in which MutS leaves the mismatch by bidirectional movement along the helix in a reaction that depends on ATP-hydrolysis by the DNA-bound protein.

Other studies on nucleotide modulation of MutS homologue–DNA interaction have relied on use of small DNAs of 20 to 200 bp in size. Nucleotide-free forms of bacterial MutS or eukaryotic MutSα bind such heteroduplexes with affinities in the nanomolar to hundred nanomolar range, depending on buffer conditions and the nature of the mismatch.[68,170,176,232,243,246,254,284] The specificity of this interaction relative to homoduplex controls is on the order of 10–20-fold. *E. coli* MutS•ADP and human MutSα•ADP complexes bind heteroduplex DNA with an affinity similar to that of the nucleotide-free protein,[243,283,284] although the specificity of the interaction is somewhat higher due to partial suppression of homoduplex binding.[284] It is important to note, however, that specificity values obtained in such experiments may significantly underestimate the true specificity of MutS homologue–DNA interaction. Recent studies indicate that binding of MutS homologues to DNA termini may contribute significantly to their apparent affinity for the linear duplexes routinely used for affinity and specificity determination.[91,234,285]

While ADP has little effect on the affinity of bacterial MutS or eukaryotic MutSα for heteroduplex DNA, heteroduplex affinity is reduced in the presence of ATP•$Mg^{2+}$ or nonhydrolyzable ATP analogues,[80,130,169,176,232,243,248,254,281,283,286,287] although substantial mismatch specificity is retained under conditions that support ATP hydrolysis.[80,92,170,234,281,284] Analysis of bacterial MutS under conditions where triphosphate hydrolysis is blocked indicates different behavior in this regard. While significant mismatch specificity is retained under low salt conditions in the presence of AMPPNP•$Mg^{2+}$, or ATP (no $Mg^{2+}$), this specificity is abolished at physiological ionic strength.[284]

MutS and MutSα complexes with short heteroduplexes (prepared in the absence of nucleotide or in the presence of ADP) undergo rapid dissociation upon challenge with ATP•

$Mg^{2+}$, ATPγS·$Mg^{2+}$, or AMPPNP·$Mg^{2+}$.[92,232,243,248,285] Examination of the fate of radiolabeled ADP in [$^3$H]ADP·MutSα and [$^3$H]ADP·MutS complexes upon DNA binding has demonstrated that bound ADP is exchanged for ATP upon heteroduplex binding,[91,233] although homoduplex DNA also promotes ATP for ADP exchange at 20−30% of the rate observed with heteroduplex.[19,91,246]

The mechanism of triphosphate-promoted dissociation from short heteroduplexes has been clarified by placement of physical barriers at ends of the linear DNAs. MutS and MutSα dissociate rapidly from heteroduplex substrates with biotin tags at both termini upon challenge with ATP·$Mg^{2+}$, but dissociation is blocked if the terminal biotins are bound to streptavidin.[232,233,238,284,288,289] Similarly, DNA structures such as four-way junctions and hairpins and protein·DNA complexes containing the Lac repressor or IHF, when strategically placed near substrate termini, also inhibit the ATP-induced release of heteroduplex-bound MutS/MutSα.[90,92,285,290] Thus, under conditions permissive for ATP hydrolysis, the dissociation of a MutS homologue from a short heteroduplex is presumed to occur at DNA ends. This effect, which has been interpreted in terms of movement of the MutS homologue from the mismatch and along the helix contour, led to the initial suggestion that MutS homologues may form a clamp-like structure about the helix.[232,233]

The formation of long-lived complexes on end-blocked DNAs in the presence of ATP·$Mg^{2+}$ raises questions concerning the hydrolytic requirements for this effect. However, attempts to resolve this question have yielded contradictory results. In one study, challenge of end-blocked MutSα·heteroduplex complexes with ATPγS or AMPPNP failed to yield long-lived complexes.[232] Similar results were obtained upon ATP challenge of such complexes with a mutant form of MutSα that binds ATP but is defective in hydrolysis.[288] These findings led to the conclusion that ATP hydrolysis by the DNA-bound MutSα is necessary to yield a mobile complex on DNA with the protein dissociating directly into solution if hydrolysis is blocked. However, a third study has demonstrated formation of the long-lived and presumably mobile complex upon ATPγS challenge of end-blocked MutSα·heteroduplex complexes.[233] Preformed MutSα·heteroduplex complexes used in the former two studies were prepared in the absence of nucleotide, while those used in the latter were prepared in the presence of ADP. It is not clear whether this experimental difference accounts for the differing results obtained in the three studies.

The work summarized above has led to two models for ATP-dependent movement of MutS homologues along the helix, both of which have significant shortcomings. The electron microscopy studies of Allen et al. led to a model in which MutS movement along DNA is dependent on ATP hydrolysis by the DNA-bound protein.[231,232,245] The shortcoming of this model is that it is difficult to reconcile with the modest rate of ATP hydrolytic turnover by MutS homologues. An alternate molecular switch-sliding clamp model for MutS homologue movement has been proposed by Fishel and colleagues on the basis of two types of observations:[19,91,233,246] (i) the finding that under some conditions, ATPγS challenge of end-blocked MutSα·heteroduplex complexes results in production of a long-lived, mobile complex, and (ii) the demonstration that binding of the ADP·MutSα complex to heteroduplex DNA can be accompanied by ATP exchange for ADP. This two-state model envisions MutSα as a molecular switch in which exchange of ATP for ADP upon mismatch binding releases the protein from the mispair with the ATP form of the protein free to diffuse along the helix as a sliding clamp.[233,243] This proposal has two limitations. Implicit in this model is the assumption that only two nucleotide-bound states are highly populated, the ADP or ATP forms. However, as discussed above, there is excellent evidence that ADP·MutS·ATP and ADP·MutSα·ATP complexes are also highly populated. This model also posits that formation of the ATP-bound sliding clamp form of the protein is sufficient to recruit a MutL homologue and activate downstream repair activities (see below). However, the mismatch dependence of ADP/ATP exchange is only 3−5-fold,[19,91,246] which is insufficient to account for the known specificity of activation of downstream repair functions.[82,93,108,182]

A hybrid proposal has been described that attempts to reconcile the shortcomings of these two models. This proposal invokes two DNA binding sites in the functional form of a MutS homolog, a clamp site through which DNA may freely diffuse and a latch site, which serves as a reflecting barrier against which DNA diffuses through the clamp site. Because nucleotide occupancy is postulated to control the open/closed state of the latch, this model would support directional movement over a substantial distance with limited energy input.[232] However, there is no direct evidence that supports this idea.

### 4.4. The (MutL/MutLα)·(MutS/MutSα)·Heteroduplex Ternary Complex

The (MutL/MutLα)·(MutS/MutSα)·heteroduplex complex is generally believed to be a key intermediate in mismatch repair. Binding of bacterial MutS to a mismatch protects about 20 bp from DNAseI cleavage in footprinting experiments.[68,78,90,251,291] In the presence of MutL and ATP this footprint expands to well over 100 bp, protecting DNA to both sides of the mispair.[80,92,251] It is not known whether the extended footprint is due to the binding of multiple copies MutL and MutS or wrapping of DNA about one of the proteins or whether DNA within the complex adopts an altered conformation that renders it less sensitive to nuclease attack. The striking nature of this complex is reflected in a DNA chain length requirement for its formation. Although specific (MutS/MutSα)·heteroduplex complexes are readily formed with DNAs as short as 20 bp, formation of the *E. coli* MutL·MutS·heteroduplex and the human MutLα·MutSα·heteroduplex ternary complex is most efficient with DNAs on the order of 100 bp or larger.[90,234]

In addition to footprinting methods, (MutL/MutLα)·(MutS/MutSα)·heteroduplex ternary complexes have been studied by electron microscopy, gel shift, and biosensor methods.[88,90−92,176−178,231,234,256,258,285] These studies have led to contradictory conclusions concerning the nature of the complex, and it is fair to say that it is not well understood. Although the consensus view is that ATP is required for ternary complex formation, the dynamics of the complex and ATP hydrolytic requirements for its formation are the subject of debate.

Several biosensor studies of ternary complexes in the *E. coli*, yeast, and human systems have indicated that assemblies are dynamic, undergoing rapid dissociation in the presence of ATP·$Mg^{2+}$ or in the absence of nucleotide.[88,234,258,285] These observations coupled with the finding that at least some ternary complexes can be trapped on linear heteroduplexes with physical barriers at both termini has led to the

suggestion that like MutS/MutSα, the MutL·MutS and MutLα·MutSα complexes may be capable of movement along the helix contour, such movement serving to carry a signal between the mismatch and the strand signal.[91,234] It is noteworthy in this regard that biosensor analysis of the yeast ternary assembly has suggested that dissociation occurs not only at DNA ends but from internal sites as well.[285] By contrast, a gel shift study of MutS·MutL·heteroduplex assembly has led to the conclusion that while MutS is capable of moving along the helix in the presence of ATP, MutL suppresses this movement, leading to a long-lived MutS·MutL complex that remains at or near the mispair.[90] These findings have been interpreted in terms of the DNA bending model for signaling between the mismatch and the strand signal that directs repair,[90,237] but it must be noted that the gel shift experiments on which this conclusion is based did not include homoduplex controls.[90] This is of concern because the mismatch dependence of ternary complex formation is relatively modest in the bacterial (2−8-fold);[88,258] human (3−4-fold),[234] and yeast systems (2-fold).[285]

The ATP hydrolytic requirements for ternary complex formation have also been a subject of controversy. Biosensor studies have indicated that AMPPNP and ATPγS are generally much less effective than ATP with respect to their ability to support mismatch-dependent ternary complex formation in the bacterial, human, and yeast systems.[88,234,285] Somewhat different conclusions were reached by Fishel and colleagues, who used biosensor methods to study sequential assembly of MutL·MutS·heteroduplex complexes.[91] Challenge of heteroduplex-bound MutS·ATP or MutS·ATPγS complexes with MutL in the presence of ATP or ATPγS demonstrated that MutS·ATPγS complexes were able to support MutL binding in the presence of the nonhydrolyzable nucleotide. Unfortunately, the significance of this finding is uncertain due to a lack of homoduplex controls in this biosensor study. Analyses of mutant forms of *E. coli* MutS defective in ATPase function have also led to conflicting conclusions. Using DNAse footprint assay, Worth and colleagues demonstrated that several MutS ATPase mutants (G619D and G614D) are defective in both mismatch repair and ternary complex formation.[251] A similar conclusion has been reached by Baitinger et al.[258] with MutS E694A, which binds ATP but is hydrolytically defective and fails to support mismatch- and MutL-dependent MutH activation. This biosensor analysis indicated that although MutS E694A supports modest levels of ternary complex formation, assembly of the complex is mismatch-independent.[258] By contrast, gel shift studies with MutS E694A have indicated that the protein does support assembly of the ternary complex, although the complex was not detectable by DNAse footprinting.[92] While it is difficult to reconcile the differing results and conclusions from these various studies, it is possible that some of the differences could be indicative of an ability of the MutL·MutS and MutLα·MutSα complexes to assume several forms on DNA. Indeed, inspection of the data from several biosensor studies clearly indicates multiphasic kinetics for dissociation of ternary complexes.[88,234,285] In the case of the human ternary complex, where data were fit to multiple exponentials, dissociation was described as triphasic.[234]

While examinations of MutS mutants defective in ATPase function have yielded contradictory conclusions, study of mutant forms of MutL defective in ATPase function has yielded consistent results. MutL ATPase integrity is not required for assembly of the bacterial MutL·MutS·heteroduplex ternary complex as judged either by gel shift or by footprinting methods.[91,92]

## 4.5. Transactivation of MutH and DNA Bending

DNA bending as a means for interaction of two DNA sites has extensive precedent in the transcription field. A similar mechanism has been proposed by Yang, Hsieh, and colleagues to account for the interaction of the two sites involved in mismatch repair.[90,92,237] In this proposal, MutS remains at or near the mismatch,[90] its ATPase providing a kinetic proofreading function. MutS is postulated to bind ATP after recognition of a putative mismatch. If a misrecognition event has occurred, the nucleotide is hydrolyzed and MutS dissociates from the DNA. However, if MutS resides at a bona fide mispair, ATP binding serves to verify mismatch recognition and is sufficient for recruitment of MutL and downstream repair activities.[237]

Three lines of evidence have been presented in support of this model. Although there is unanimity with respect to the conclusion that ATP binding by mismatch-bound MutS/MutSα confers mobility on the protein, a gel shift study described above has led to the suggestion that MutL recruitment leads to a stable complex that remains at or near the mispair.[90] A bending model is also consistent with the demonstration that a mismatch on one oligonucleotide duplex can activate MutS- and MutL-dependent MutH cleavage of a second duplex and that a mismatch on one arm of a four-way junction can activate MutH incision at a GATC sequence located on another arm.[90,237] Although integrity of the MutL ATP hydrolytic center is required for MutH activation in trans, hydrolytically defective MutS E694A was found to support the reaction;[237] however, subsequent studies have shown that MutS E694A is defective in its ability to support cis activation of MutH on a hemi-methylated 6.4 kbp heteroduplex that has been used to score methyl-directed repair in vitro.[258] It should also be noted that the transactivation and four-way junction cleavage reactions are quite inefficient as compared to cis MutH activation on the 6.4 kbp heteroduplex (0.0002−to 0.002 $min^{-1}$ per MutH for trans and four-way junction cleavage as compared to 0.1−0.2 $min^{-1}$ per MutH for the cis reaction on the 6.4 kbp DNA).[90,93,237]

Perhaps the most compelling evidence for the bending model has been provided by Hays and co-workers who constructed nicked circular heteroduplex DNAs with physical barriers (DNA hairpins or biotin−streptavidin blocks) located between the mismatch and the strand break that directs repair.[238,290] Analysis of the fate of such molecules in HeLa nuclear extracts demonstrated that the barriers were without significant effect on the initiation of mismatch-provoked excision at the strand break, although progress of excision through the barrier was attenuated substantially. Hence, it was concluded that signaling between the mismatch and the nick can occur when physical barriers are placed between the two DNA sites. While these experiments are fairly convincing, several caveats should be noted. The possibility that a hairpin block itself might provoke a repair response was not tested.[290] In the case of the avidin experiments, the blocks were offset from the helix by a 15-carbon linker, and the studies did not include controls addressing the possibility that the repair system or other HeLa extract activities might be capable of transient displacement of bound avidin from the DNA.

### 4.6. Comments on the Models

While the DNA bending model has several attractive features and has garnered significant experimental support, it has a major shortcoming. Analysis of the bacterial and human mismatch repair systems has demonstrated that both respond to heteroduplex orientation: the excision reaction elicited at the strand break depends on its 3′ or 5′ placement relative to the mismatch. Function in this manner requires that the repair system establish heteroduplex orientation, and it is not clear how this can be accomplished by a bending mechanism. Determination of the relative orientation of the two DNA sites would seemingly depend on the transduction of a signal along the helix contour.

It is well established that MutS homologues are capable of ATP-dependent movement along the helix, and several studies have attributed a similar mobility to MutS·MutL homologue complexes, although our understanding of this assembly is at an early stage. ATP-dependent movement in this manner could in principle serve to establish heteroduplex orientation, and this idea has received much attention in the literature. However, there is no proof that that this type of mechanism is responsible for signaling between the two DNA sites involved in mismatch repair.

A third model that could serve to establish heteroduplex orientation has received less attention in the literature. In this type of mechanism, mismatch recognition by a MutS homologue serves as a nucleation site for polymerization of a second protein along the helix.[235] In fact, yeast MutLα has been shown to cooperatively polymerize on DNA in a manner that dramatically increases with chain length.[236] Although polymerization is readily evident at modest MutLα concentrations, it is largely abolished at physiological salt concentrations in the presence of $Mg^{2+}$. Furthermore, electron microscopic visualization of bacterial MutS and MutL on heteroduplex DNA under repair conditions has failed to reveal evidence for significant polymerization.[231] Nevertheless, it may be premature to discount this type of model, because a treadmilling variation has not been excluded. For example, a mechanism in which addition of a MutL (or MutS) unit to the head of polymer chain occurs only slightly faster than dissociation from the tail would yield a polymer of only modest length. Such a mechanism would not only suffice to establish heteroduplex orientation but also confer apparent movement along the helix, as has been described in a number of the studies described above.

## *5. Mismatch Repair in the DNA Damage Response*

### 5.1. Lesions that Trigger a Mismatch Repair-Dependent Damage Response

The mammalian mismatch repair system has been implicated in the cellular response to several types of DNA damage, including lesions produced by $S_N1$ DNA methylators, 6-thioguanine, 5-fluoro-deoxyuridine, cisplatin, ultraviolet light, and several carcinogens. Recognition and perhaps processing of such lesions by mismatch repair leads to activation of damage signaling pathways, resulting in cell cycle arrest and, at high lesion load, apoptosis. The versatility of the repair system in terms of its ability to respond to a variety of base pair anomalies, including conventional mismatches, has led to the suggestion that it may function as a general sensor of DNA damage.[14,292]

The cytotoxic effects of $S_N1$ DNA methylators (e.g., *N*-methyl *N*-nitrosourea (MNU), *N*-methyl *N*′-nitro *N*-nitrosoguanidine (MNNG), temozolomide (8-carbamoyl-3-methylimidazo[5,1-*d*]-1,2,3,5-tetrazin-4(3*H*)-one), procarbazine (*N*-isopropyl-α-(2-methylhydrazino)-*p*-toluamide), and dacarbazine (5-(3,3-dimethyl-1-triazenyl)-1*H*-imidazole-4-carboxamide) are largely due to production of $O^6$-methylguanine,[293−296] which can pair with cytosine or thymine.[297,298] Although 6-thioguanine (6TG) has several modes of action,[299−302] the purine analogue is incorporated into DNA where it is subject to spontaneous methylation by *S*-adenosylmethionine, with the resulting 6-MeTG presumed to pair with cytosine or thymine.[303] 5-Fluoro-2′-deoxyuridine (FdU) can also be incorporated into DNA where it may also promote mispairing.[304,305]

Despite their intrinsic mutagenic activity, the cytotoxic effects of DNA methylators, 6-TG, FdU, and cisplatin have been exploited in their use as antitumor drugs.[294,295,304,306,307] The effectiveness of these agents is often mitigated by development of resistance, a phenomenon that has been linked to defects in mismatch repair. Association of mismatch repair defects and drug resistance has been documented in cultured cells[14,15,151,300,308−312] and mice,[313] and clinical data are beginning to appear indicating that patients with mismatch repair-deficient cancers respond poorly to treatment with at least some of these agents.[314−316]

Involvement of mismatch repair in the cytotoxicity of DNA methylators and cisplatin was initially described in *E. coli*. Although wild-type *E. coli* are resistant to these compounds, strains deficient in the Dam methylase are sensitive to killing by these agents, and this sensitivity is reversed by introduction of *mutS* or *mutL* mutations.[13,317,318] Thus, DNA methylator and cisplatin killing occur in a MutS- and MutL-dependent manner, but only when DNA lacks the GATC modification that directs repair. This killing effect has been attributed to production of double-strand breaks[77] via cleavage of both strands of an unmethylated GATC site by activated MutH.[93,319] The pathways leading to cell death in mammalian cells differ from those in *E. coli*, but as discussed below, a common theme in the two systems is the dependence on lesion recognition by a MutS homolog.

The human lymphoblastoid cell line MT1 was isolated from TK6 cells by one-step selection for high-level resistance to the cytotoxic effects of MNNG and exhibits a mutator phenotype.[320] This cell line is defective in strand-specific mismatch repair due to genetic inactivation of both *MSH6* alleles and consequent MutSα deficiency.[14,130,132] A similar correlation between methylation tolerance and inactivation of MutSα was observed in cultured hamster cells[15] and mouse *MSH2*$^{-/-}$ ES cells.[308] Subsequent studies have shown that MutLα is also required for the cytotoxic response elicited by DNA methylators and that MutSα and MutLα defects also confer resistance to 6-TG, cisplatin, and FdU.[151,154,300,305,309−311,321,322] It is noteworthy that MutS$\beta$ is not required for the response to DNA methylator damage.[130,323,324]

The mismatch repair system has also been implicated in the cellular response to lesions produced by several chemical carcinogens, as well as those produced by ultraviolet irradiation. Deficiency of MutSα or MutLα renders human cells resistant to apoptotic killing by *N*-acetoxy-2-acetyl-2-aminofluorene (AAAF) and benzo[*a*]pyrene-7,8-dihydrodiol-9,10-epoxide (B[*a*]PDE).[325] AAAF derivatizes the C-8 of deoxyguanosine, whereas B[a]PDE attacks the exocyclic

amino group of this base. Evidence for involvement of mismatch repair in the ultraviolet response was initially obtained in *E. coli* where recombinational rescue of UV-irradiated $\lambda$ bacteriophage was shown to be dependent on a functional mismatch repair system.[21,326] The response of mismatch repair-deficient mammalian cells to UV irradiation has not been extensively examined; however, several recent studies have demonstrated that a functional MSH2 is required for a normal apoptotic response to UVB radiation in mouse cells and whole animals. In the latter case, it has also been shown that *MSH2*-null mice develop UVB-induced skin tumors at reduced levels of radiation exposure.[327,328]

## 5.2. Mediation of the Mismatch Repair-Dependent Damage Response

Key elements of the eukaryotic DNA damage response are the G1 and G2 checkpoints.[329] Exposure of normal mammalian cells to $S_N1$ DNA methylators, 6-TG, or cisplatin leads to cell cycle arrest at the G2 checkpoint,[300,320,330−332] and cells with a high load of DNA methylator lesions proceed from G2 arrest into apoptosis.[323,331,332] In the case of DNA methylator damage, these cellular responses are bypassed in mismatch repair-deficient cells,[14,320] and they are presumably attenuated for other classes of damage where cytotoxicity depends on a functional repair system.

Two models have been proposed to account for function of mammalian MutSα and MutLα in the damage response. One model invokes translesion DNA synthesis upon replication fork encounter of damage within the template strand with the resulting base pair anomaly activating the mismatch repair system. Since action of this system is restricted to the new strand, the offending lesion cannot be removed, leading to abortive turnover of newly synthesized DNA.[320] Intermediates occurring during such futile cycling could serve as a scaffold for recruitment of damage signaling kinases. This model is consistent with the finding that DNA methylator treatment of repair-proficient cells results in G2 arrest in the second cell cycle after methylator exposure, implying occurrence of replicative bypass of template damage.[320,331] The alternate model posits recruitment of MutSα, MutLα, and perhaps other activities to the site of a damaged base with this complex sufficient to initiate damage signaling in the absence of excision repair.[14,16,24]

Both of these models invoke action of MutSα and MutLα during the earliest steps of the damage response. Several lines of evidence support this view. DNAs containing $O^6$-methylguanine ($O^6$-MeG) are processed by the human mismatch repair system in nuclear extracts.[324,333,334] Furthermore, human MutSα has been shown to specifically recognize a variety of lesions produced by the damaging agents described above including $O^6$-MeG−C and O6-MeG−T base pairs, the cisplatin 1,2-intrastrand GpG cross-link (but not the nontoxic 1,3 transplatin intrastrand cross-link), AAAF, and B[a]PDE lesions, as well as cyclobutane pyrimidine dimers and [6,4]-photoproducts produced by ultraviolet irradiation.[16,17,20,325] In the case of cisplatin cross-links and UV photoproducts, which are normally processed by nucleotide excision repair, presence of a noncomplementary base (e.g., a T opposite a G in the cisplatin 1,2 GpG cross-link) enhances MutSα affinity for the lesion.[22,23,25,335] Similarly, an activity present in mismatch repair proficient cells has been identified that binds 6-MeTG−T base pairs. Inasmuch as this activity is lacking in *MSH2*$^{-/-}$ or *MSH6*$^{-/-}$ cells, it has been presumed to be MutSα.[301,303] In fact, duplexes containing 6-TG or 6-MeTG are bound by MutSα with a hierarchy of affinities: 6-TG−T > G−T > 6-MeTG−C > 6-MeTG−T ≫ G−C > 6-TG−C (ref 16, C. Koh, S. Dunham, C. Baitinger, and P. Modrich, unpublished).

Action of MutSα and MutLα during the earliest steps of the damage response implies that the repair proteins would act upstream of damage signaling kinases that trigger the cellular DNA damage response. This possibility has been addressed using p53 as an in vivo substrate to score activation of damage signaling kinase response to DNA methylator damage. Treatment of human cells with MNU or MNNG results in p53 phosphorylation at Ser-15 and Ser-392. Phosphorylation at both sites was shown to be dependent on functional MutSα and MutLα, implying that these activities act upstream of the kinases responsible for these p53 modifications.[324] Residues Ser-15 and Ser-392 of p53 are preferred substrates for ATM and ATR kinases,[336,337] which play key roles in the DNA damage response via parallel pathways involving phosphorylation-dependent activation of the downstream checkpoint kinases Chk1 (by ATR) and Chk2 (by ATM).[329] Indeed, exposure to $S_N1$ methylators or 6-TG has been variously reported to involve MutSα- and MutLα-dependent phosphorylation of Chk1, Chk2, or both, providing further evidence for ATM or ATR involvement or both in this process.[338−345] Additional evidence for functional involvement of MutSα and MutLα in ATM and ATR activation has been provided by observations suggesting direct interactions between the mismatch repair proteins and kinases involved in these damage-signaling cascades. Thus, MSH2 has been reported to interact with ATR, MLH1 with ATM, and MSH2 with Chk2.[339,346] In addition to ATM- and ATR-dependent signaling pathways, the mitogen-activated protein kinase p38 has also been implicated in damage signaling triggered by $S_N1$ DNA methylator lesions. Protein p38 is activated in response to DNA methylator damage in an MLH1-dependent fashion, and down-regulation of p38 by siRNA silencing or by use of pharmacological inhibitors abrogates G2 checkpoint arrest.[347]

The p73 tumor suppressor regulates a p53-independent apoptotic pathway.[348,349] Wang and colleagues[350,351] have demonstrated a p73 requirement for an MLH1-dependent response to cisplatin damage. Levels of p73 are elevated upon cisplatin treatment of mismatch repair-proficient cells, an effect that is accompanied by activation of the tyrosine kinase c-Abl, which phosphorylates p73, thereby increasing its half-life.[350] These responses are defective in MLH1-deficient cells. This signaling system may involve physical interaction between PMS2 and p73 because association between the MutLα subunit and p73 is enhanced upon cellular exposure to cisplatin.[351] Possible involvement of p73 and c-Abl in the damage response to $S_N1$ methylators has not been reported.

The simplest interpretation of these findings is that the mismatch repair system provides a critical lesion sensing function that permits mammalian cells to respond appropriately to several classes of DNA damage. As noted above, two models have been proposed to explain function of the repair system in this regard: (i) activation of damage signaling kinases via a lesion bypass mechanism that triggers MutSα- and MutLα-dependent excision and (ii) MutSα- and MutLα-dependent assembly of a damage signaling complex at the site of a lesion that may reside in nonreplicating DNA. These models are not necessarily mutually exclusive, and experimental support is available for both ideas. Cell cycle arrest in the second G2 following $S_N1$ DNA methylator

exposure and the demonstration that cisplatin and pyrimidine dimer compound lesions are preferentially recognized by MutSα are most consistent with the lesion bypass model. In addition, the finding that ExoI deficiency confers partial resistance to 6-TG in mouse cells suggests involvement of excision in the response to this compound (E. Avdievich and W. Edelmann, unpublished). On the other hand, mouse cells that harbor a G674A missense mutation within the ATPase domain of MSH2 display differential defects in mismatch repair and the cisplatin-induced damage response. In contrast to *MSH2*-null cells, which are defective in mismatch repair and cisplatin-resistant, cells harboring the G674A allele are repair defective but remain cisplatin-sensitive,[352] suggesting that excision is not required for the damage response elicited by cisplatin. Elucidation of the molecular details responsible for MutSα- and MutLα-dependent damage signaling should further clarify these issues.

These studies also have implications with respect to genetic stabilization afforded by the mismatch repair system. Virtually all of the agents considered in this section, including the antitumor drugs mentioned above, are mutagens and include several well-known carcinogens. Somatic mutations are believed to contribute in a major way to tumor development,[353−356] and the cancer predisposition associated with mismatch repair defects has generally been attributed to the failure to correct DNA replication errors. However, the damage signaling functions of mismatch repair provide another mechanism by which the system can effectively stabilize the genome. It is reasonable to assume that inactivation of the damage-signaling functions of the pathway also contribute to the cancer predisposition associated with mismatch repair defects.[325]

## 6. Other Functions of Mismatch Repair

### 6.1. Mismatch Repair and the Fidelity of Genetic Recombination

MutS and MutL homologues modulate the outcome of mitotic and meiotic recombination events in a number of ways, and comprehensive descriptions of these effects can be found in several recent reviews.[32,33,357] Such effects are generally not well understood in terms of mechanism. Consequently, this section will be restricted to consideration of MutS and MutL homologue function in the suppression of recombination between quasi-homologous DNA sequences, so-called homeologous recombination, a phenomenon for which mechanistic information is available that can at least partially account for the observed biological effects.

The genomes of virtually all organisms harbor multiple copies of related sequence elements; however, the fact that chromosome rearrangements are rare implies that recombination between such quasi-homologous sequences is infrequent. The puzzling nature of this stability became evident with the demonstration that the activities responsible for recombinational strand transfer readily promote exchange between DNAs that differ significantly at the sequence level.[358,359] Initial clues to the nature of this barrier to exchange between diverged sequences was provided by *E. coli* studies, which demonstrated that inactivation of MutS or MutL dramatically increases the frequency of homeologous exchanges between quasi-homologous sequences.[360−363] The biological importance of this mismatch repair function is dramatically illustrated by the work of Radman and colleagues.[360,363] *E. coli* and *Salmonella typhimurium* do not normally exchange genetic information; however, the presence of a *mutS* or *mutL* mutation within one of these species allows it to incorporate DNA content from the other into its genome. In other words, the repair system is a major determinant responsible for definition of the species barrier in bacteria. Subsequent studies with yeast and mammalian cells have demonstrated similar functions of eukaryotic MutS and MutL homologues in the suppression of homeologous recombination.[32,33] In the case of human cells, MutSα or MutLα deficiency increases the rate of gene duplication 50−100-fold, a genetic destabilization effect attributed to illegitimate recombination that may contribute to the cancer predisposition conferred by mismatch repair deficiency.[364]

Several molecular features of the anti-recombination activity of *E. coli* MutS and MutL were revealed by in vitro analysis of the effects of the two proteins on RecA-mediated strand transfer as a function of the degree of homology of the DNAs involved in the reaction. Although without effect on RecA-mediated strand transfer reactions in which the two participating DNAs were identical (i.e., reactions in which both DNAs are derived from bacteriophage fd or both from M13), MutS and MutL block homeologous strand transfer between fd and M13, which differ by 3% at the sequence level.[365] High concentrations of MutS alone are sufficient to suppress fd−M13 strand exchange, but MutL dramatically potentiates this effect at reduced MutS concentrations. Mutant forms of MutS, which retain mismatch recognition activity but lack ATPase function due to amino acid substitutions within the Walker A motif, also suppress fd−M13 strand transfer. However, MutL fails to enhance this effect, suggesting that MutS ATP hydrolysis is required for MutL function in this manner.[251] Examination of strand transfer intermediates produced during fd−M13 strand exchange has demonstrated that strand transfer initiates in the presence of MutS and MutL and that the mismatch repair proteins block the branch migration step of strand assimilation.[365] This suggests that MutS and MutL are able to access mismatched base pairs within early strand transfer intermediates and that this serves to block branch migration. Inasmuch as RecA strand transfer products reside within a nucleoprotein filament,[366] MutS and MutL are apparently able to access mispairs within this structure, but the mechanism by which this occurs is not clear. It is noteworthy that yeast genetic studies support the view that MutS and MutL homologues intervene in homeologous exchange by blocking the branch migration step of strand transfer. Thus, gene conversion tracts produced by exchange between quasi-homologous sequences in mismatch repair-deficient cells are substantially longer than those observed in otherwise isogenic repair-proficient cells.[367,368]

The homeologous branch migration intermediates that are trapped by MutS and MutL have been postulated to undergo a disassembly reaction,[365] but the fate of such structures has not been established. It also is pertinent to note in this regard that the fate of mispairs within a homeologous strand transfer intermediate need not be the same as that for mismatches that arise at the replication fork. For example, the fate of the MutS•MutL•mismatch complex that occurs within a recombination intermediate could be dictated by its presence within the context of the RecA nucleoprotein filament.

### 6.2. Mismatch Repair and Triplet Repeat Instability

Trinucleotide repeat expansion is the cause of several common neurodegenerative diseases such as myotonic

dystrophy, Huntington's disease, fragile-X syndrome, and Friedreich's ataxia.[369] The expansion of CTG•CAG, CGG•CCG, and GAA•TTC repeat tracts located in the vicinity of specific genes has been attributed to the propensity of these sequences to form unusual secondary structures during DNA replication, recombination, and repair.[369,370] In a very surprising finding, Jaworski et al. demonstrated that long CTG•CAG repeat tracts (>100 repeats) expand and delete less frequently in *E. coli* strains deficient in MutS, MutL, or MutH as compared to otherwise isogenic repair-proficient strains.[371] Detailed examination of the mismatch repair dependence of CTG•CAG repeat instability has revealed two distinct classes of events. Whereas small expansions and deletions (1−5 repeats) are prevented by mismatch repair, the occurrence of large changes (>5 repeats) depends on the functional integrity of the MutHLS pathway.[372−375]

Subsequent studies with mice have led to similar conclusions. Somatic and germline expansions at CAG•CTG repeat loci within the Huntington's gene are dependent on a wild-type *MSH2* gene.[376−378] Similarly, CTG•CAG repeat expansions within a myotonic dystrophy protein kinase (DMPK) transgene depend on functional *MSH2* and *PMS2* loci,[379,380] the latter requirement implicating MutLα. Interestingly, instability is reduced substantially in *MSH3*-null mice but is enhanced in an *MSH6*-null background,[381] suggesting that MutS$\beta$ plays a major role in triplet repeat destabilization. Although several models have been proposed to account for the mismatch repair-dependent destabilization of triplet repeats,[371,374,377,378,380] the molecular events responsible for expansion in this manner remain to be established.

### 6.3. Mismatch Repair and Generation of Immunoglobulin Diversity

Antibody diversity is achieved through genetic alteration of immunoglobulin genes by V(D)J recombination, class switch recombination, and somatic hypermutation (SHM) during clonal expansion of B-lymphocytes.[382] The variable regions of immunoglobulin genes in B-lymphocytes are targets for a mutagenic process that results in a highly elevated mutation rate of $10^{-3}-10^{-5}$ per base pair per cell generation.[383] While mismatch repair defects enhance mutability in a typical somatic cell, genetic inactivation of *MSH2, MSH6, PMS2, MLH1*, or *EXOI* reduces the recovery of one class of mutation that occurs within immunoglobulin gene variable regions as a consequence of SHM.[384−393] Thus, although mismatch repair defects do not alter the incidence of mutations occurring at G•C base pairs within variable region hotspots, they significantly reduce the recovery of alterations that occur at A•T base pairs.

To account for these and other observations, Neuberger, Milstein, and their colleagues have proposed a two-stage model for SHM.[387] Stage I mutations are largely restricted to G•C base pairs within the RGYA/T consensus. The first stage of somatic hypermutation requires activation-induced cytidine deaminase (AID), which deaminates cytosines to yield G−U mispairs.[394] These G−U mismatches are postulated to have several alternative fates. They may be fixed as transition mutations by replication, or upon action of uracil DNA glycosylase[5] the abasic site product may serve as template for lesion bypass DNA synthesis, resulting in transition and transversion mutations.[395] As discussed below, a third possibility involves recognition of these mismatches by MutSα[23,396]

Subsequent stage two mutations are primarily localized to A•T base pairs within the T/AA consensus and are dependent upon integrity of the mismatch repair system. As compared to wild-type mice, the SHM spectrum of MSH2- or MSH6-deficient animals is dramatically shifted toward occurrence at G•C base pairs.[387,390,392] *ExoI*$^{-/-}$ animals also exhibit a bias against mutations occurring at A•T base pairs,[393] and modest alterations of the mutation spectrum have been described for MLH1- or PMS2-deficient mice.[388,397,398] These findings suggest a mutagenic process occurring at A•T base pairs that is dependent on MutSα, ExoI, and to a lesser extent, MutLα,[395] which may be initiated by MutSα recognition of G−U mispairs.[23,396] The mechanism by which mismatch repair dependent A•T mutations arise is unclear, although the limited MutLα dependence of stage II mutagenesis at A•T base pairs is reminiscent of the in vitro studies described above, which have demonstrated that mismatch-provoked excision directed by a 5′-strand break can proceed in a MutLα-independent manner. It has been suggested that repair of the ensuing gap in an error prone manner may account for mutations targeted to A•T base pairs.[395,396,399] Indeed, recent studies have suggested that MutSα may interact with DNA polymerase $\eta$, thereby stimulating its activity as it synthesizes across gaps generated by G−U provoked, MutSα-dependent excision.[396,399]

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