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DNA MISMATCH REPAIR AND CANCER

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1. ABSTRACT

DNA mismatch repair (MMR) is an important genome caretaker system. It ensures genomic stability by correcting mismatches generated during DNA replication and recombination and by triggering apoptosis of cells with large amounts of DNA damage. Protein components responsible for these reactions are highly conserved through evolution, and homologs of bacterial MutS and MutL, which are key players in the initiation steps of both the strand-specific mismatch correction and MMR-dependent apoptotic signaling, have been identified in human cells. Inactivation of genes encoding these activities leads to genome-wide instability, particularly in simple repetitive sequences, and predisposition to certain types of cancer, including hereditary non-polyposis colorectal cancer.

2. INTRODUCTION

It is well accepted that cancer is caused by genetic mutations (1, 2). In addition to the mutations that are induced by exogenous DNA-damaging agents, mutations can also arise from mismatched base pairs generated during normal DNA metabolism. To safeguard the integrity of the genome, cells possess multiple mutation avoidance systems including base excision repair (BER), nucleotide excision repair (NER), and mismatch repair (MMR). Whereas BER and NER repair damaged or modified bases, MMR is known to correct mispairs that are derived from DNA replication and recombination. In bacteria, the importance of MMR in maintaining genomic stability was demonstrated ~ 30 years ago with the observation that defects in this pathway lead to elevated levels of spontaneous mutability (3, 4). Inactivation of MMR in human cells also results in a mutator phenotype, and is associated with both hereditary and sporadic cancer development. Whereas the ability of the MMR system to correct DNA mismatches has been considered the primary mechanism by which it contributes to genomic stability, recent studies indicate that the MMR system also contributes to genomic stability by mediating programmed cell death in response to certain DNA damaging agents. The apoptotic function of the MMR system eliminates

severely damaged cells from growing, thereby preventing tumorigenesis.

The rapid increase in knowledge about MMR in yeast and humans that has occurred in the last decade has led to our current understanding of the molecular mechanisms of MMR in eukaryotic cells. The focus of this review will be on human MMR and its role in cancer avoidance. However, as an introduction to MMR in human cells, the *Escherichia. coli* system will be discussed briefly. Readers interested in the details of the genetics and biochemistry of MMR in humans and other organisms are referred to a number of excellent reviews (5-18, also see T. Wilson's review in this issue of encyclopedia).

3. MECHANISM OF MISMATCH REPAIR (MMR)

3.1. Overview of the methyl-directed MMR pathway in E. coli

The best characterized MMR pathway is the *E. coli* methyl-directed repair system, which involves eleven protein activities, including MutS, MutL, MutH, DNA helicase II (MutU/UvrD), four exonucleases (ExoI, ExoVII, ExoX, and RecJ), single-stranded DNA binding protein (SSB), DNA polymerase III holoenzyme, and DNA ligase (14, 19-21). Among these proteins, MutS, MutL, and MutH are the three components that initiate the repair process and are believed to act only in the MMR pathway. MutS, a 97 kDa protein, is the mismatch recognition protein that binds to both base-base mismatches (22) and small nucleotide insertion/deletion (ID) mispairs (23) as a homodimer. In addition to mismatch recognition, MutS possesses an intrinsic ATPase activity (24), which is essential for the MMR function since defects in the ATPase activity results in a dominant negative mutator phenotype (25). Recent studies demonstrate that MutS has to interact simultaneously with ATP and a mismatch in order to activate the down-stream repair events, suggesting a role for the MutS ATPase activity in proofreading of the MMR reaction (26). Thanks to the elegant work by the laboratories of Yang, Hsieh, and Sixma, several structures of MutS protein-DNA complexes have been recently determined by X-ray crystallography (26-28). These structures have confirmed that MutS binds to a mismatch as a homodimer, a result consistent with previous biochemical studies (29, 30). Interestingly, the mismatch binding site is comprised of non-homologous domains from each protein monomer, indicating an asymmetric use of residues from each subunit (27, 28). Hence, the MutS homodimer appears to bind to a mismatch as a virtual heterodimer, a characteristic adopted by eukaryotic MutS homologs (see below). For a detailed discussion of these MutS structures, see references (8, 12, 31).

Although MutL is absolutely required for MMR, none of the known enzymatic activities essential for the reaction has been assigned to the protein. Nevertheless, MutL has been shown to physically interact with MutS to enhance mismatch recognition. Additionally, both MutS and MutL are required for the recruitment and activation of MutH, an endonuclease that incises the daughter DNA strand (29, 32-35). Because MutL also is required for the loading of helicase II at the MMR initiation site (36-39), the protein appears to generally act as a molecular chaperone to facilitate the assembly of functional MMR protein complexes (14, 40). Like MutS, MutL functions as a homodimer and possesses an ATPase activity (34). Structural and functional studies have indicated that ATP binding and hydrolysis modulate the conformation and oligomeric state of MutL (9, 41). Mutations (e.g. E32K) in the ATP binding domain lead to a dominant negative mutator phenotype both *in vivo* and *in vitro* (42, 43). The E32K-substituted MutL protein fails to support MMR *in vitro* and to activate MutH. Because the mutant protein is capable of interacting with MutS, MutH, and helicase II (43), it is conceivable that the MutL E32K forms non-productive protein complexes that block subsequent steps in the MMR pathway (41, 43). Conversely, MutL mutant proteins that are defective in ATP hydrolysis but proficient in ATP binding can activate MutH but cannot stimulate MutH in response to a mismatch or MutS, suggesting that ATP hydrolysis by MutL is essential for mediating activation of MutH by MutS (26).

In *E. coli*, DNA is methylated at the N6 position of adenine residues in GATC sequences, but the newly replicated daughter strand is transiently unmethylated in these sequences. It is these hemimethylated GATC sequences that allow repair to be targeted to the newly synthesized daughter strand, where the incorrect base is located. The mechanism by which this occurs involves MutH, a protein that functions as a monomer and belongs to the family of type-II restriction endonucleases (9, 44). After its recruitment and activation by MutS and MutL in the presence of ATP, MutH specifically cleaves the transiently unmethylated daughter strand within the GATC sequence (26, 45). The nicking of the unmethylated strand marks it for excision. Interestingly, excision of the daughter strand can occur without the requirement for GATC hemimethylation and MutH if mismatched DNA contains a pre-existing strand break (19).

It is well accepted that repair initiation begins with the binding of a MutS homodimer to the mismatch. Next, a methyl group that is either upstream (5') or downstream (3') of the incorrect base is identified by the concerted action of the initiation factors MutS, MutL, MutH, in the presence of ATP. The details of how the first initiation step leads to the second are extremely sketchy and have been subject to hypothesis; three models have been proposed in the literature (Figure 1). In the translocation model, Modrich and colleagues (5, 29) propose that ATP promotes bi-directional translocation of MutS by drawing flanking DNA toward the protein complex after its initial binding to a mismatch, yielding an α-like loop structure (Figure 1A). The formation of the MutS-mediated α-loop structure provides a simple mechanism for interaction of the two important DNA sites: mismatch and methyl group. In the second model (the sliding clamp or molecular switch model), Fishel and co-workers (46, 47) propose that mismatch recognition by MutS or its homologs provokes ADP$\rightarrow$ATP exchange. The nucleotide swap results in a conformational transition in MutS that allows it to act as a diffusible clamp to slide

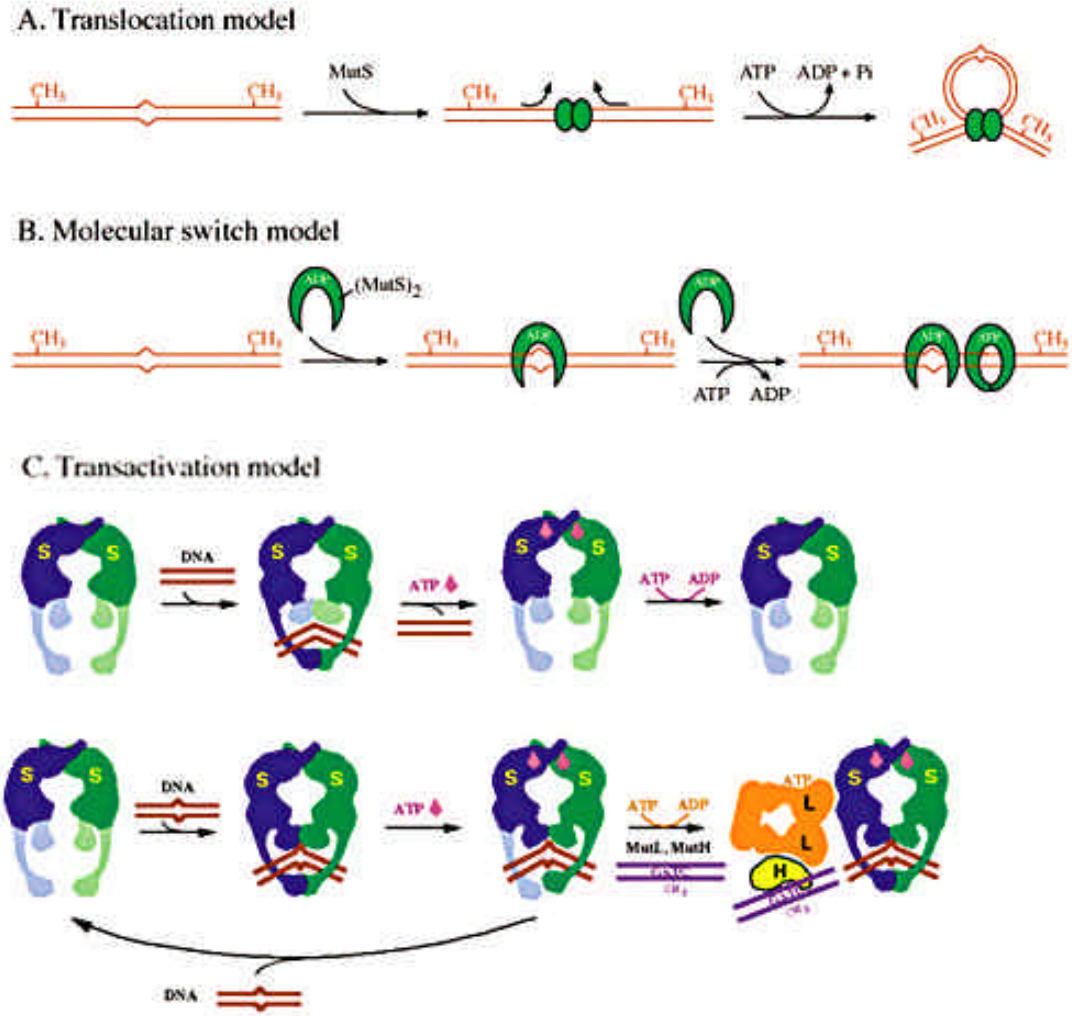

Figure 1. Models for MMR initiation. A, Translocation model. The MutS dimer first recognizes and binds to the mismatch. ATP reduces the mismatch binding affinity of MutS and ATP hydrolysis drives bi-directional translocation of MutS to form an α-like loop structure of DNA. B, Molecular switch model. MutS is suggested to be present in either an ADP-bound form (open "Pacman" form) or an ATP-bound form ("closed ring" form). Binding of the ADP-bound form of MutS to a mismatch stimulates the exchange of ADP for ATP. The nucleotide switch results in a conformational change of MutS and promotes the ATP-MutS complex to diffuse along the DNA helix. ATP is not hydrolyzed in this diffusible complex. C, Trans-activation model. Binding of ATP enables MutS to release a homoduplex DNA (upper) or to continue to bind a heteroduplex DNA through a loosening interaction (lower). In the presence of MutL, MutH, and the hemi-methylated GATC sequence, the bound ATP molecules allow the MutS-mismatch complex to form a repair complex of the three proteins with two DNA recognition sites and initiate mismatch repair. The ATP bound by MutS is shown in purple, and the ATP hydrolyzed by MutL during the trans-activation is shown in orange. The translocation model is reproduced from Junop *et al.* (26) with permission.

along the DNA helix (Figure 1B). While the translocation model and the sliding clamp model differ in respect to how MutS approaches the strand discrimination signal, both suggest that MutS has to moves away from the mismatch site before activating the repair process. Recently, Yang and her colleagues (26) have demonstrated that MutS, along with MutL, ATP, and a mismatch-containing heteroduplex DNA, can activate the MutH endonuclease regardless whether or not the hemimethylated GATC sequence is in the same molecule as the mismatch. Since the crystal structures do not support a translocation of MutS away from the mismatch site, these investigators proposed a third model (26), referred to as trans-activation model (see Figure 1C). In this model, MutS utilizes ATP binding to distinguish between homoduplex and heteroduplex by reducing its binding affinity to DNA. MutS releases DNA if it binds to a homoduplex or if MutL and/or MutH is not available even through a heteroduplex is bound. In the presence of MutL, MutH, hemimethylated GATC sequence, the bound ATP molecules direct MutS to assemble a repair initiation complex among the three Mut proteins and the two DNA recognition sites, thereby initiating the MMR reaction.

Table 1. MMR components and their functions

E. coli	Human	Function
$(MutS)_2$	hMutSα (MSH2-MSH6)[a] hMutSβ?(MSH2-MSH3)	DNA mismatch/damage recognition
$(MutL)_2$	hMutLα (MLH1-PMS2)[a] hMutLβ (MLH1-PMS1) hMutLγ (MLH1-MLH3)	Molecular matchmaker/chaperone?
MutH	?[b]	Strand discrimination
UvrD	?[b]	Unwinding DNA helix
ExoI, ExoVII, ExoX, RecJ	ExoI	Removing mispaired base
Pol III holoenzyme	Pol δ, PCNA	DNA re-synthesis
SSB	RPA	Protecting template DNA from degradation
DNA Ligase	?[b]	Nick ligation

[a] Major components in cells. [b] Not yet identified.

The strand break created by MutH at a GATC site of the unmethylated strand serves as a starting point for the excision of the mispaired base. In the presence of MutL, helicase II loads at the nick (36, 37) and unwinds the duplex from the nick towards the mismatch, revealing a ssDNA region of the un-nicked strand to which SSB binds to prevent its attack by nucleases. Depending on the position of the strand break relative to the mismatch, ExoI or ExoX (3'→5' exonuclease), or ExoVII or RecJ (5'→3' exonucleases) degrades the nicked strand from the nicked site (the GATC site) up to and slightly past the mismatch (48). The resulting single-stranded gap undergoes repair resynthesis and ligation by the DNA polymerase III holoenzyme, SSB, and DNA ligase.

The *E. coli* methyl-directed, MutHLS-dependent MMR pathway possesses the following features. First, it is a strand-specific; i.e., the repair is only targeted on the newly synthesized strand where the incorrect base is positioned. Second, repair is bi-directional; i.e., excision can proceed from the nick in either a 5'→3' or 3'→5' direction (20, 48). Finally, the system has a fairly broad substrate specificity, being able to process both base-base mismatches and a variety of ID mispairs (5, 49). All of these properties require functional MutS, MutL, and MutH proteins. For example, the bi-directional capability of the system, which is achieved through the action of several exonucleases, requires functional MutS and MutL. Because the mechanism of the bacterial MMR pathway appears to be well conserved through evolution, it serves as a paradigm for the MMR process in eukaryotic cells.

3.2. Overview of MMR in human cells

Human cells possess an MMR pathway that is homologous to the *E. coli* methyl-directed, MutHLS-dependent system (50, 51). As shown in Table 1, these two pathways have many similarities. Like the *E. coli* pathway, the human system efficiently repairs both base-base mismatches and small ID mispairs (50-56). Human cells, however, have a broader substrate specificity than *E. coli*, as evidenced by their ability to efficiently repair C-C mismatches and ID mispairs larger than 7 but smaller than 16 nucleotides (55, 56), also see Figure 2), which cannot be corrected by the *E. coli* system (49). The strand discrimination signal in human cells does not appear to involve methylation (50), but it does involve recognition of and targeting to a strand containing a pre-existing strand break (50, 51, 57). This suggests that like the *E. coli* reaction, the strand-specific repair in human cells is also nick-directed. As in *E. coli*, the human MMR reaction is capable of bi-directional processing of a mispaired base. Regardless of whether the strand break is 5' or 3' relative to the mismatch, mismatch-provoked excision always proceeds on the shortest path from the nick to the mismatch (57).

Based on the hypothesis that the *E. coli* and human MMR systems are homologous, a search has been undertaken to identify the protein components involved in human MMR. Indeed, human homologs of the *E. coli* MutS and MutL proteins, two of the three known MMR-specific *E. coli* functions, have been identified and shown to be required in the human MMR pathway. In addition, most of the proteins involved in the steps of excision and resynthesis have recently been identified, which include exonuclease activity hEXOI (58-63), single-strand DNA binding protein RPA (64, 65), proliferating cellular nuclear antigen (PCNA) (66-71), and DNA polymerase δ (72). A yeast DNA helicase, RRM3, has been postulated to be involved in MMR based on its interaction with PCNA (73), but its human equivalent has not been identified. The discovery of the similarities in substrate specificity, component activities, and repair mechanism between *E. coli* and human MMR has greatly advanced our current understanding of the human MMR pathway.

A major difference between these two systems is that while the *E. coli* MutS and MutL proteins are homodimers, the human counterparts function as heterodimeric complexes. Among the three human MutS homologs (hMSH2, hMSH3, and hMSH6) involved in strand-specific MMR, hMSH2 interacts with hMSH6 or hMSH3 to form hMutSα(?54) or hMutSβ (56, 74), respectively. Like the MutS protein in *E. coli*, both hMutSα and hMutSβ possess an intrinsic ATPase activity (75-79), which plays a critical role in MMR initiation (46, 76, 79). In addition, these heterodimers are responsible for mismatch recognition. hMutSα preferentially recognizes base-pair mismatches and ID mispairs of 1 or 2 nucleotides whereas hMutSβ preferentially recognizes ID mispairs

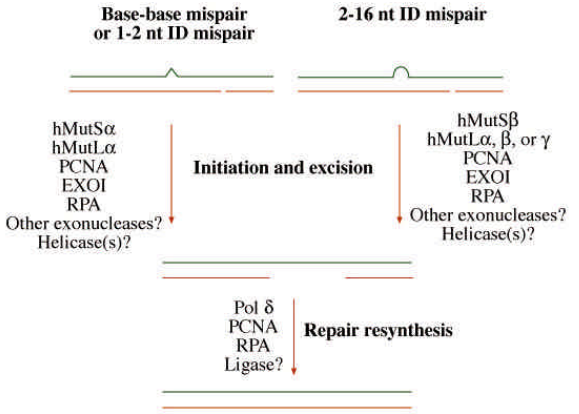

Figure 2. Schematic diagram of MMR in human cells. The human MMR system can bi-directionally process all eight base-base mismatches and 1-16 nt ID mispairs. The repair process in each case involves repair initiation, excision, and resynthesis. Except for mismatch recognition, where hMutSα and hMutSβ are required to distinguish specific substrates as indicated, activities required for the remaining steps of the reaction are believed to be the same for the processing of base-base mismatches and ID mispairs.

(Figure 2). Although hMutSβ is capable of binding to up to 24 ID mispairs, only those containing 16 nt or less can activate the ATPase activity of the protein to initiate MMR (80). This observation is supported by *in vitro* repair data demonstrating that the largest ID mispairs that the human MMR system can process is 16 nucleotides (55).

Four human MutL homologs, hMLH1, hMLH3, hPMS1, and hPMS2, have been identified (81-84). hMLH1 interacts with hPMS2, hPMS1, or hMLH3 to form three heterodimeric complexes (84-88), designated hMutLα, hMutLβ, or hMutLγ, respectively. While the function of hMutLβ is not clear at this time, hMutLα is required for MMR and hMutLγ appears to be involved in meiosis (89). Like the MutL protein in *E. coli*, hMutLα possesses an ATPase activity (90-93), and defects in this activity result in loss of MMR function (92-94). The crystal structures of the N-terminal 40 kDa fragment of hPMS2 and its complexes with ATPγS and ADP have been resolved (95). An alignment of the peptide sequences between hPMS2 and MutL reveals considerable sequence homology within the conserved ATPase domain, and suggests that hPMS2 may possess significant structural homology to MutL in this region. If so, then the crystal structures suggest that the MutL homologs in humans, like MutL in *E. coli*, may promote protein-protein interactions in MMR through conformational changes induced by ATP binding. However, unlike the *E. coli* MutL, whose ATPase activity requires protein dimerization, the monomeric form of hPMS2 is capable of both ATP hydrolysis and DNA binding (95).

The interaction between hMutSα and hMutLα in the presence of a mismatch substrate *in vitro* has been demonstrated (67, 96). Additionally, other proteins required for human MMR have been identified based on their ability to interact with one or both of these proteins. For example, PCNA has recently been shown to be required for MMR in eukaryotes. PCNA is known as a protein cofactor required for the efficient replication of DNA by polymerase δ and ε; new functions for this protein in the cell, however, are emerging (97, 98). The involvement of PCNA in MMR was first suggested when it was observed that PCNA interacts with eukaryotic Mut homologs, as demonstrated by a yeast two-hybrid system (66) and by co-immunoprecipitation (67). Subsequently, a PCNA interaction domain was identified in both MSH6 and MSH3 (68, 70, 71). PCNA is required for both MMR initiation (66, 67, 69) and resynthesis (67). While the role for PCNA in the step of resynthesis is justified by the fact that PCNA is an accessory factor of DNA polymerase δ, how PCNA functions at the step of initiation is (at present) not clear. A recent study has indicated, however, that PCNA may play an important role in MMR initiation by transferring MutSα to the mismatch site (99). The identification of eukaryotic EXOI as a required MMR protein was made by virtue of its ability to interact with MSH2 (58, 61, 100) and MLH1 (62) in both yeast and human cells. Although the eukaryotic EXOI contains only 5'→3' exonuclease activity, it is required in removing mismatched bases from both the 5'→3' and the 3'→5' orientations (63, 101). Based on previous findings in *E. coli* and yeast (21, 100), it is reasonable to believe that the human MMR reaction may require more than one exonuclease. Suprisingly, cells derived from *EXO*I-knockout mice are defective in the repair of base-base and single-base ID mismatches in both 5' and 3' nick-directed repair (101). Therefore, the mechanism of the excision step of the mammalian MMR remains unclear. The role of human single strand DNA binding protein RPA has recently been demonstrated (65). RPA protects the template DNA strand from degradation after a single strand gap is generated as a result of repair excision. RPA also functions to facilitate MMR resynthesis (65), which is conducted by replicative polymerases including pol δ (72, also see Table 1 and Figure 2).

4. MMR DEFICIENCY AND COLORECTAL CANCER

MMR has long been postulated to be an important cellular mechanism that maintains genomic stability. However, loss of MMR function was not implicated as a cause of cancer susceptibility until 1993, when MMR defects were found to be associated with hereditary non-polyposis colorectal cancer (HNPCC) and a subset of sporadic colon cancers. HNPCC, or Lynch syndrome, is a heritable autosomal dominant disease, which is defined by the presence of colorectal cancer in at least three family members in two successive generations, with one affected member having been diagnosed at less than 50 years of age (102). HNPCC accounts for 4-13% of all colorectal cancer (103), one of the most common forms of neoplasia in Western populations (104). In addition to colon cancer, individuals from HNPCC families are at increased risk to develop cancers of the endometrium, ovary, stomach, urinary tract, brain, and other epithelial organs (105, 106). Although HNPCC was suspected to be a

heritable disease almost a century ago (107), the molecular pathogenesis of this disease was not established until 1993.

4.1. Microsatellite instability in HNPCC and sporadic colorectal cancer

An understanding of the molecular basis of HNPCC developed from a genetic linkage analysis of HNPCC kindreds completed over a decade ago, which demonstrated the presence of a disease locus at p15-16 of chromosome 2 (108). It was hypothesized that the genetic basis for HNPCC could be the loss of a tumor suppressor gene at this locus. To test this hypothesis, a team lead by Vogelstein and de la Chapelle, employed microsatellite markers to determine if allelic losses occurred in the p15-16 region of chromosome 2. This strategy was undertaken because tumor suppressor genes, if mutated, often undergo loss of heterozygosity, leading to allelic losses in the area of the disease locus. These investigators found no allelic losses in this HNPCC locus; instead, insertion or deletion mutations at repetitive sequences were found in 11 of the 14 tumors examined (109). These unexpected mutations were evident in each di- and trinucleotide repeat (microsatellite) tested and were referred to as RER^+ (replication error positive) (109). In addition, these investigators also found a subset of sporadic colon cancers with a similar phenotype, but occurring at a much lower incidence (6 out of 46) (109). At the same time, two other groups led by Perucho (110) and Thibodeau (111) independently reported microsatellite alterations in 12-15% of sporadic colon cancers, and that these alterations appeared to be a genome-wide phenomenon. Taken together, these findings suggest that microsatellite instability (MSI) in HNPCC and a subset of sporadic colorectal cancers is a genome-wide phenomenon and may be caused by a common mechanism. Although the genetic basis of HNPCC remained unidentified at that time, these studies provided an important clue as to the mechanism of its action.

4.2. MMR defects are the genetic basis of HNPCC

4.2.1. The linking of MMR defects with MSI tumors: a brief history

The identification of MSI in colorectal cancers received a great deal of attention from cancer investigators as well as from geneticists and biochemists working in the field of DNA MMR, because the mutational fingerprint of HNPCC tumors is similar to that found in MMR-deficient cells. At that time, the following points had been established: (i) loss of MMR function leads to genome-wide base-base substitutions as well as frameshift mutations (5, 112); (ii) MMR proteins recognize and process ID mispairs (23); and (iii) repetitive dinucleotide sequences undergo frequent contractions or expansions in MMR-deficient bacterial cells (113, 114). Therefore, the hypothesis was made that the genetic defects in HNPCC involve a loss of MMR function.

Several groups tested this hypothesis using different approaches. First, Petes and colleagues (115) examined the stability of poly(GT) tracts in yeast strains with either a single or double knockout of *MSH2*, *MLH1*, or *PMS1*. All mutants (both single and double mutants) exhibited 100- to 700-fold elevated levels of tract instability involving insertion or deletion of 2-4 repeated units (115). This study strongly supported an association of MMR defects with the HNPCC syndrome. Second, Kolodner and co-workers and Vogelstein and co-workers independently searched for human MMR homolog genes and determined their association with HNPCC kindreds. Both groups reported the cloning of the *hMSH2* gene using PCR products of degenerate primers derived from two highly conserved regions of the known bacterial MutS and yeast MSH proteins (116), and located the gene on the p arm of chromosome 2 (117, 118). Additionally, germ-line mutations of *hMSH2* were indeed identified in HNPCC families (118). Third, Modrich and co-workers (52) and Kunkel and co-workers (53) took a biochemical approach and examined the MMR-proficiency of tumor cells derived from HNPCC and sporadic colorectal cancers with MSI. Both laboratories demonstrated that cell extracts of these tumor cells are completely defective in repair of base-base and ID mispairs. These *in vitro* biochemical studies provided definitive evidence supporting the hypothesis that MMR defects are the genetic basis of HNPCC.

4.2.2. Germline mutations of MMR genes in HNPCC

Immediately following the mapping of the first HNPCC-linked locus to chromosome 2p (108), Lindblom *et al.* (119) identified a second locus linked to HNPCC predisposition at p21-23 of chromosome 3. In a remarkably short period of time after the cloning of *hMSH2* (117, 118), three human MutL homolog genes (*hMLH1*, *hPMS1*, and *hPMS2*) were cloned (81-83). Liskay and coworkers (81) identified and mapped the *hMLH1* gene to the second HNPCC locus, and missense germ-line mutations in *hMLH1* were found in a family with a history of HNPCC (81). At the same time, Vogelstein and co-workers searched a human cDNA database and also identified the *hMLH1* gene (83). In addition, they reported two additional human *MutL* homolog genes, *hPMS1* and *hPMS2*, which are located on the q and p arms of chromosome 2 and 7, respectively (82). Germ-line mutations of each human *MutL* homolog were found in HNPCC kindreds, with defects in *hMLH1* present in the majority of the HNPCC cases (82, 83).

Since the initial identification of HNPCC-linked genes, HNPCC kindreds have been extensively screened for mutations in each of these genes (120-133). It is clear now that mutations of *hMSH2* and *hMLH1* account for ~ two-thirds of all HNPCC kindreds tested while mutations in *hPMS1* and *hPMS2* are rare (82, 134). Recently, mutations of hMSH6 have been found at a high incidence in atypical HNPCC families (135). No germ-line mutations in *hMSH3* have yet been identified in HNPCC patients. The observed distribution of mutations of these genes in HNPCC is consistent with the relative importance of their functional roles in MMR, as judged by the fact that the protein products of *hMSH2* and *hMLH1* are obligatory components of all MMR-associated hMutS and hMutL heterodimers known to date.

Recently, a fourth human *MutL* homolog gene, *hMLH3*, has been identified (84), but its involvement in

Table 2. Phenotypes of MMR-deficient knockout mice

Gene	MSI	Tumor	Fertility	Reference
MSH2	Yes	Lymphoma, GI, skin, and other tumors	Yes	157, 158, 282
MSH3	Yes	GI tumors	Yes	163, 164
MSH6	Low instability in dinucleotide repeats	Lymphoma, GI and other tumors	Yes	161, 163, 164
MLH1	Yes	Lymphoma, GI, skin, and other tumors	No	159, 160
PMS1	Mononucleotide repeats only	None	Yes	162
PMS2	Yes	Lymphoma and sarcoma	Male only	156, 162
MLH3	Yes	Not available	No	89
EXOI	Mononucleotide repeats only	Lymphoma	No	101

tumorigenesis of HNPCC is controversial. While two groups failed to link *hMLH3* to HNPCC (136, 137), a third group reported germ-line mutations of the gene in some patients with HNPCC (138). A controversy also exists in terms of whether or not germline mutations of *hEXOI* are linked to HNPCC. Wu *et al.* (139) reported several germline mutations of *hEXOI* in HNPCC families, but the same alterations were also identified in normal populations by Jagmohan-Changur *et al*. (140), suggesting that the alterations may be polymorphism. Nevertheless, recombinant hEXOI proteins carrying these alterations have been shown to either lack the exonuclease activity or display a reduced capacity to interact with hMSH2 (141). Interstingly, a very recent study has demonstrated that like the *MSH2*- or *MLH1*-knockout mice, mice defective in *EXOI* are deficient in strand-specific MMR and susceptible to cancer (101).

4.3. Restoration of MMR to colorectal tumor cells by MMR gene products

The most convincing evidence that the HNPCC syndrome is caused by MMR defects may come from the biochemical studies of this disease. Biochemical assays of extracts prepared from a number of cell lines that were derived from HNPCC and sporadic tumors with MSI have clearly demonstrated that these cells are deficient in strand-specific MMR (52-54, 142-148). Further characterization of these cell lines has defined at least two *in vitro* complementation groups (53, 54, 144), which led to the isolation of hMutLα and hMutSα (54, 85). Purified hMutLα or hMutSα restores strand-specific MMR to nuclear extracts derived from colorectal tumor cell lines that are defective in hMLH1/hPMS2 or hMSH2/hMSH6 (54, 85), respectively.

Strong evidence supporting the concept that MMR genes are crucial to genomic stability was also provided by chromosome or gene transfer experiments (149-153). Boland and colleagues reported that the transfer of human chromosome 3 carrying the wild-type *hMLH1* gene to an *hMLH1*-deficient colorectal tumor cell line restores MMR to the cell line (149). Similarly, human chromosome 2 containing both the *hMSH2* and *hMSH6* genes, can complement MMR defects in *hMSH2*- and *hMSH6*-deficient tumor cell lines (150, 151, 154). Restoration of MMR to cell lines defective in *hPMS2*-, *hMLH1*-, or *hMSH6* has also been achieved by introduction of the corresponding genes into these lines (152, 153, 155). Most strikingly, the transfected genes or chromosomes also stabilize simple repetitive sequence in the host cells. These studies further confirm that the MMR system plays an essential role in the maintenance of genomic stability and show the potential of gene therapy in the treatment of HNPCC.

4.4. Mouse models of human MMR defects

To understand the relationship between MMR defects and tumorigenesis in HNPCC, mice with a knockout mutation in each MMR gene have been developed (89, 156-164). Although most of them display a typical mutator phenotype (e.g., exhibiting MSI) and a predisposition to develop cancer, it is surprising that none of these MMR-deficient mice developed colon cancer as in HNPCC. Instead, a significant number of these animals developed lymphomas, particularly those deficient in *MSH2*, *MLH1*, and *PMS2* (Table 2). In addition, these knockout animals also develop gastrointestinal tumors, skin neoplasms and/or sarcomas (Table 2, for reviews see Refs. 18, 165, 166).

The *MSH2*-deficient mice develop normally and both male and female mice were fertile (157, 158). Although there seem to be no distinguishable differences between wild-type mice and heterozygous $MSH2^{+/-}$ mice, the homozygous $MSH2^{-/-}$ mice have a much shorter lifespan, with 50% dying by six months of age (158). The $MSH3^{-/-}$ mice, although defective in repair of small insertion/deletion mispairs, exhibit a tumor susceptibility phenotype that is similar to wild-type mice (164). The lack of a significant cancer phenotype in the *MSH3*-knockout mice provides an explanation as to why germ-line mutations of the human *MSH3* gene have not been identified in HNPCC patients. The $MSH6^{-/-}$ mice display a tumor spectrum similar to $MSH2^{-/-}$ mice and usually develop tumors within their first year of life (161, 163). $MSH6^{-/-}$ mice do not show the typical MSI phenotype that is detected in $MSH2^{-/-}$ mice. Interestingly, the mutations and pathology observed in $MSH6^{-/-}$ mice seem to be similar to that observed in atypical HNPCC cases with hMSH6 mutations, which are characterized by a late cancer onset (>60 years of age) and low rates of MSI (135).

Mice defective in *MutL* homologs (*MLH*) share many of the $MSH^{-/-}$ characteristics in terms of cancer spectrum and genomic instability. However, a striking feature that is unique in MLH mutant mice (except *PMS1* mutants) is that they are infertile (89, 159, 160). While both male and female mice defective in *MLH1* or *MLH3* are sterile (89, 159, 160), only male *PMS2*-knockouts are sterile (156). Unlike all of the MMR-deficient mice described above, mice deficient in *PMS1* gene show no instability in repeat sequences except for a small mutation rate in mononucleotide repeats (162). Most

strikingly, the *PMS1*-knockout mice do not develop any tumors (162).

Knockout mice for *EXOI* have recently been developed (101). Despite the fact that EXOI is one of the exonucleases involved in *E. coli* and yeast MMR (21, 100), cells derived from *EXOI*$^{-/-}$ mice are hypermutable at both the *HPRT* locus and a mononucleotide repeat marker, and are defective in strand-specific MMR; and *EXOI*$^{-/-}$ animals exhibit reduced survival and increased susceptibility to the development of lymphomas (101). Like *MLH1*$^{-/-}$ and *MLH3*$^{-/-}$ mice, both male and female *EXOI*$^{-/-}$ animals are sterile (101, also see Table 2).

In summary, although MMR-deficient mice do not develop colon cancer and there are variations among these knockouts in terms of cancer spectra and mutator phenotypes (18, 165-167), these studies certainly support the view that MMR defects lead to genomic instability and eventually to cancer, as originally proposed based on studies of the HNPCC syndrome. In addition, the phenotypes of the individual MMR gene knockouts are basically consistent with the role of their gene products in MMR, as characterized by previous biochemical and genetic studies.

4.5. MMR defects caused by epigenetic modifications

As described above, mutations in MMR genes that account for the hypermutable phenotype are associated with the HNPCC syndrome and a subset of sporadic colorectal cancers with MSI. However, in a significant fraction of sporadic colon tumors displaying MSI, no identifiable mutations have been found in known MMR genes (168-170), suggesting that a different mechanism may be involved in causing MSI in these cases. Surprisingly, the search for this mechanism has linked these tumors again to MMR defects. This time, however, an epigenetic factor, methylation, is responsible for suppressing the expression of MMR genes (147, 171-176).

Kane *et al.* (171) demonstrated that hypermethylation of the *hMLH1* promoter is correlated with a lack of hMLH1 expression in several sporadic colon tumors and cell lines that are free of mutations in the *hMLH1* gene. Therefore, hypermethylation is probably a common mode of MMR gene inactivation in sporadic cancer (171). Since then, hypermethylation of the *hMLH1*gene has been extensively studied (147, 172-175). According to the Bethesda guidelines (177), sporadic tumors can be classified into three types based on their MSI status in five sets of microsatellite markers: microsatellite stable (MSS, instability observed in none of the 5 markers), low-frequency MSI (MSI-L, instability observed in one of the 5 markers), and high-frequency MSI (MSI-H, instability observed in 2 or more markers). It has been reported that more than 95% of MSI-H tumors are due to loss of expression of hMLH1 (178). Interestingly, almost all MSI-H tumors that do not have a detectable mutation within the *hMLH1* gene demonstrate hypermethylation in the *hMLH1* promoter (174, 175). Very recently, hypermethylation of the *hMLH1* promoter has been shown in an HNPCC patient, who does not have germ-line mutations in any of known MMR genes (179). In contrast, hypermethylation of the *hMSH2* gene has not been observed in tumors with MSI (174).

To determine the nature of the hypermethylation of the *hMLH1* promoter in these MSI tumors, two independent research groups, led by Markowitz and Herman, treated several tumor cell lines deficient in hMLH1 expression due to hypermethylation in the *hMLH1* promoter with the demethylating agent 5-aza-deoxycytidine (147, 173). This treatment successfully restored hMLH1 protein expression in all tumor cells that lack hMLH1 expression due to a methylated *hMLH1* promoter. The expression of hMLH1 is associated with the presence of unmethylated *hMLH1* alleles (147, 173). More importantly, extracts derived from 5-aza-deoxycytidine-treated cells are capable of performing strand-specific MMR (173). These experiments indicate that in addition to genetic defects, epigenetic modification of MMR genes can also result in a mutator phenotype.

5. MMR DEFICIENCY AND NON-COLORECTAL CANCER

5.1. Microsatellite instability in non-colorectal cancer

The identification of MSI in HNPCC patients in 1993 led to the elucidation of the molecular pathogenesis of this disease. Since then, a great body of work has been published demonstrating that MSI is also associated with a wide variety of non-HNPCC and non-colonic tumors (for detailed reviews see Refs. 177, 180-182). These tumors include endometrial, ovarian, gastric, cervical, breast, skin, lung, glioma, prostate, bladder, leukemia, and lymphoma. Because these studies were carried out using different microsatellite markers and employing different numbers of samples, it is not surprising that the observed mutation rates vary from study to study, and in some cases are not in agreement with one another. To standardize MSI studies internationally, the Bethesda guidelines were developed and suggest that at least five loci should be used in MSI studies; instability in one of the five loci will be scored as" MSI-L" and instability in two or more loci will be scored as "MSI-H" (177).

Based on the Bethesda guidelines, non-colorectal tumors also exhibit the MSI-H and MSI-L phenotypes (177). Most of the sporadic endometrial and gastric tumors, lung cancers and lymphomas display a high level of MSI in many markers (183). Some tumors demonstrate greater instability in one marker than another. In this regard, tumors with MSI can be divided into two groups: one that displays elevated instability at mono- and di-nucleotide markers and, to a lesser degree, at larger repeat markers, and a second group that displays elevated instability only at specific larger repeat markers, such as tri- and tetranucleotide repeats. Endometrial and gastric tumors usually belong to the first group, while bladder, lung, head and neck cancers belong to the second group (177, 184).

5.2. MMR deficiency in sporadic non-colorectal cancer

The presence of MSI in sporadic non-colonic tumors stimulated a search for somatic mutations in MMR

genes, particularly the *hMSH2* and *hMLH1* genes in these tumors. Although somatic mutations in each of the MMR genes in sporadic cancer and cell lines with MSI have been documented (145, 168, 170, 171, 176, 185-194), the major mechanism underlying MMR deficiency in sporadic cancers was shown to be epigenetic silencing, i.e. promoter hypermethylation of MMR genes, especially the *hMLH1* gene. Hypermethylation of the *hMLH1* promoter has been demonstrated in sporadic endometrial, gastric, and breast cancers (175, 194-196). Biochemical studies have shown that cell lines derived from sporadic endometrial, ovarian, prostate, and bladder cancers are defective in strand-specific MMR (53, 144, 145, 197, 198). These findings suggest that MMR defects are a likely cause of non-colonic sporadic cancer with MSI, although other mechanisms may also be involved in causing the MSI mutator phenotype.

6. MMR DEFICIENCY AND INACTIVATION OF GENES CRITICAL FOR CELLULAR GROWTH CONTROL AND GENOMIC STABILITY

Despite evidence described above that MMR genes function like tumor suppressor genes, the MMR pathway is a mutation avoidance system, or a "caretaker" system (199). It is anticipated that loss of MMR function will affect stability of many genes, including critical gatekeeper genes (e.g. tumor suppressor genes) and caretaker genes (e.g. DNA repair genes). Because of technical limitations, it is impossible at this time to assess the impact of MMR-deficiency on a genome-wide basis and to identify all mutations that accumulate due to MMR-deficiency. However, using MSI analysis, it is possible to readily detect frameshift mutations in genes that contain simple repeat sequences within their coding regions, which in most cases lead to truncated proteins.

Markowitz *et al.* (200) reported that mutations in the type II transforming growth factor-β receptor (*TGF-b RII*) gene are associated with sporadic colorectal cancer cells that are defective in MMR. These mutations are all frameshift mutations and occur either in a six-bp GTGTGT repeat or in an $(A)_{10}$ mononucleotide repeat (200). In each case, the frameshift mutation results in a mutant form of the *TGF-b RII*. Subsequent studies have demonstrated that frameshift mutations of simple repeat tracts in the *TGF-b RII* gene are common in colorectal tumors with MSI (201-207). Similar mutations of *TGF-b RII* have also been observed in many types of MSI cancer, including gastric cancer (208-211), glioma (212), uterine cervical cancer (213), squamous carcinoma of the head and neck (214), ulcerative colitis-associated neoplasm (215), and sporadic cecum cancer (216). It is known that TGF-β RII is required for transduction of the TGF-β growth inhibitory signal to suppress epithelial cell growth. The loss of TGF-β RII function in tumors with MSI represents a crucial mechanism by which cells may escape from growth control. The targeted mutations in the simple repeated sequences of the *TGF-b RII* gene may be characteristic of the genome-wide alterations that are expected in MMR deficient cells.

In addition to *TGF-b RII*, somatic frameshift mutations of mononucleotide runs have been documented in several genes critical for cellular growth in tumors with MSI. These genes include the apoptosis gene *Bax* (217-224), insulin-like growth factor 2 receptor *IGF2-R* (221, 225-227), transcription factor *E2F-4* (210, 228), tumor suppressor genes *APC* (229-231) and *PTEN* (196, 225, 232-234), and DNA repair genes *hMSH3* (189, 235), *hMSH6* (189-192, 235), *Mre11* (236), and *MBD4/MED1* (237). All of these genes are crucial either for cellular growth control or for genomic stability; and the inactivation of any of these genes could be a key mechanism by which tumors with MSI become neoplastic. Therefore, the potential impact of loss of the tumor suppressor function of MMR is not only relevant to HNPCC, but to virtually all types of cancer.

7. MMR, THE DNA DAMAGE RESPONSE, AND APOPTOSIS

7.1. MMR deficiency and drug resistance

While MMR is well known for its role in correcting biosynthetic errors, other important roles for MMR proteins are being recognized, one of which is to mediate programmed cell death (or apoptosis) of cells with heavily damaged DNA (for a review see Ref. 238). The recognition of the apoptotic role for the MMR system developed from research on how chemical and physical DNA damaging agents cause cell death.

Treatment of cells with chemical DNA-damaging agents such as the alkylating agents *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG), temozolomide, or procarbazine leads to increased amounts of cell death. For this reason, these cytotoxic agents are often used in chemotherapy to destroy rapidly growing tumor cells. It has been found almost universally that while cells that are proficient in MMR are sensitive to these agents, cells that are deficient in MMR are more resistant to killing by these agents. The phenomenon was initially observed in *E. coli* MMR mutants in 1980s (239-241), and it also applies to human cells. In fact, the first human MMR mutant cell line, MTI, was derived from the TK6 lymphoblastoid cell line by selection with a high dose of MNNG (242). The MT1 cell line was subsequently found to harbor a mutation in hMSH6 (243) and to be defective in strand-specific MMR (112). Whereas MMR-deficiency can be acquired following treatment with the alkylating agent MNNG, MMR-deficient cell lines derived from HNPCC and MSI tumors are also resistant to alkylating agents. For example, an hMLH1-defective colorectal tumor cell line is resistant to killing by MNNG, but it becomes sensitive to the agent when receiving a wild-type copy of hMLH1 by transfer of chromosome 3 (149, 244). Similarly, cells defective in other MMR genes also confer resistance to alkylating agents (143, 157, 245-251). Similar results were also obtained with other drugs or chemicals (252-256).

Drug resistance has also been found to be associated with changes in expression profiles of MMR genes, which results in a loss of MMR function. For example, treatment of the HL60 leukemia cell line with methotrexate (MTX), a frequently used chemotherapeutic drug for cancers (257), induces over-expression of the *hMSH3* gene (258, 259). The primary target for MTX is dihydrofolate reductase (DHFR), a key enzyme that

catalyzes the reduction of dihydrofolate to tetrahydrofolate in a reaction essential for nucleotide metabolism. It has been well documented that human tumor cells acquire resistance to MTX due to the amplification of a chromosomal region containing the *DHFR* gene (260-262), leading to elevated expression of DHFR. Interestingly, the *DHFR* gene shares a promoter with a second gene (now known as *hMSH3*) that is transcribed in the opposite direction (263, 264). Instead of enhancing MMR activity, over-expression of hMSH3 renders the MTX-treated cells to be defective in MMR (258, 259), which is believed to be responsible for the MTX-resistant phenotype observed in HL60 and other tumor cells. This is because both hMSH3 and hMSH6 interact with hMSH2 to form hMutSβ and hMutSα, respectively, and over-expression of hMSH3 allows the protein to capture almost all cellular hMSH2 so that little hMutSα can be formed. At the same time, uncomplexed hMSH6 is subject to degradation (54, 258). In normal conditions, cellular hMutSα:hMutSβ ratio is ~ 10:1, and cells with no hMutSα or lower ratio of hMutSα:hMutSβ are hypermutable (54). These observations clearly indicate that drug resistance is closely associated with MMR deficiency caused either by genetic mutations or by changes in expression profiles of MMR genes.

7.2. MMR proteins promote DNA damage-induced cell cycle arrest and apoptosis

The distinct responses to DNA-damaging agents between MMR-proficient and MMR-deficient cells have stimulated research on how the cell responds to DNA damage. It has been found that cells proficient in MMR undergo growth arrest at the G2-phase of the cell cycle after treatment with MNNG or 6-thioguanine (6-TG), but the G2-phase arrest was not observed in cells deficient in MMR under the same treatment (153, 244). A recent study by Brown *et al.* (265) has indicated that the MMR system is also required for activation of the S-phase checkpoint in response to ionizing radiation. In addition, cytotoxicity of DNA damaging agents is associated with apoptosis, and the apoptotic response only occurs in MMR-proficient cells (250, 255, 266-268).

Recent studies have indicated that both p53 and the related protein p73 are implicated in MMR-dependent apoptosis. Upon treating cells with DNA damaging agents, phosphorylation of p53 and/or p73 has been noted in MMR proficient cells, but not in cells defective in either hMutSα or hMutLα (269, 270). ATM and c-Abl appear to be the kinases that phosphorylate these proteins during the damage response (265, 270). Physical interactions between MMR proteins (e.g., hMutSα and hMutLα) and proteins involved in the DNA damage-signaling network (e.g., ATM and p73) have been recently identified (265, 271). These observations indicate that MMR-dependent apoptosis in response to DNA damage involves a signaling cascade.

7.3. MMR-mediated apoptosis eliminates potentially tumorigenic cells

The molecular events involved in the MMR-dependent apoptotic response have not yet been established. However, increasing evidence suggests that the apoptotic signaling is initiated by MMR proteins. Two models have been proposed to account for MMR-mediated apoptosis (Figure 3). One model proposes that a repetitive attempt by MMR to remove a DNA adduct in the template DNA strand causes cell death. DNA adducts in the template strand can pair with appropriate bases or lead to mispairs during DNA replication. hMutSα, along with hMutLα, recognizes these unusual base pairs as "mismatches" and provokes a strand-specific MMR reaction. However, because MMR is always targeted to the newly synthesized strand, adducts in the template strand cannot be removed and thus the unusual base pairs reform upon DNA resynthesis during repair. As a result, the repair cycle may be perpetually reinitiated. Such a futile repair cycle may signal cells to switch on apoptotic machinery. A second model suggests that the death signal could come from the binding of hMutSα/hMutLα to DNA adducts in the replication fork and/or the unwound DNA helix. These protein-adduct complexes may block DNA transactions such as replication, transcription, and repair, and could be recognized as a signal for cell cycle arrest and apoptosis. Both models provide an explanation for the selective growth advantage of cells that are defective in MMR. Strong support for these models is provided by the fact that MutS and its eukaryotic homologs are capable of recognizing a variety of DNA adducts caused by DNA damaging agents (reviewed in Ref. 238), such as MNNG (272), cisplatin (272-275), environmental chemical carcinogens (255, 276), oxidative free radicals (277, 278), and ultraviolet (UV) light (274, 279).

As described above, the repair function of the MMR pathway prevents mutations from building up in the genome by correcting mispairs. The apoptotic function of the pathway, however, maintains genomic stability by promoting apoptosis of cells with severely damaged DNA. Normally, base excision repair and nucleotide excision repair pathways are responsible for repair of DNA damage induced by physical and chemical agents. However, when these pathways are not available or there is too much damage to be repaired, the genome is in danger of accumulating a large number of mutations, which are tumorigenic (1, 2). Therefore, eliminating these damaged cells from the body would be beneficial. The MMR system is capable of activating apoptotic machinery to eliminate these pre-tumorigenic cells from growing by promoting apoptosis. The inability of this system to commit damaged cells for apoptosis is thought to be a molecular basis for cancer development (238, 280).

8. CONCLUSION AND PERSPECTIVES

The seminal discovery of MMR defects in HNPCC and sporadic cancers with MSI in 1993 has greatly stimulated investigations on MMR and its role in preventing cancer. Almost all human homologs of the *E. coli* MMR components have now been identified and characterized by both genetic and biochemical approaches. It is now known that MMR maintains genomic stability through both its repair and apoptotic functions. The repair function of the system is to correct base-base and ID

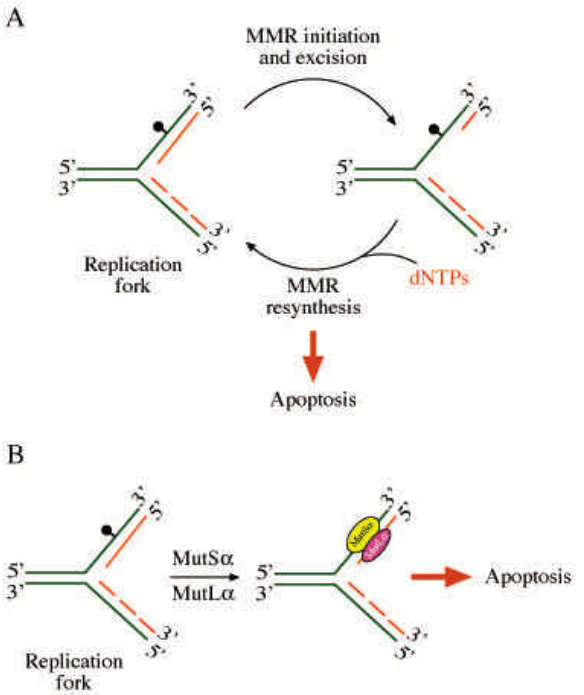

Figure 3. Models for MMR-dependent apoptosis in response to DNA damage. A, The futile repair cycle model. The adducted base (solid circle) could pair with an appropriate base or lead to a mispair during DNA replication. This abnormal structure can be recognized by hMutSα (hMSH2-hMSH6) and provoke a strand-specific MMR reaction. However, since MMR can be only targeted to the newly synthesized strand (red line), the offending adduct in the template strand (black line) cannot be removed, and will initiate a new cycle of MMR upon repair resynthesis. Such a futile repair cycle stimulates an interaction between MMR proteins and apoptotic transducers (e.g., p73 and p53) to activate the apoptotic machinery. B, The blockage model. The bindings of hMutSα/hMutLα to a DNA adduct in the replication fork and/or the unwound DNA helix could block DNA transactions such as replication, transcription, and repair. The blockage promotes MMR proteins to interact with apoptotic transducers to switch on apoptotic machinery.

mispairs that are generated during normal DNA metabolism, ensuring a mutation-free genome. The apoptotic function of MMR, however, is to signal genetically damaged cells to commit to apoptosis, removing pre-tumorigenic cells from the body. Therefore, loss of MMR function, by either genetic mutation or epigenetic modification, will lead to a mutator phenotype and predispose a cell to become neoplastic.

While MMR-mediated apoptosis is important for tumor suppression, the drug resistance property of MMR-deficient cells raises concerns for cancer chemotherapy. First, certain widely used clinical drugs, such as temozolomide, procarbazine, and cisplatin, are expected to be harmful for patients with tumors caused by MMR defects. This is because these drugs will preferentially kill the patient's normal proliferating tissue via drug-induced apoptosis, rather than the tissue of the tumor. Therefore, chemotherapy should be used with caution for tumors with MMR defects. Second, since some tumor cells can acquire MMR deficiency upon exposure to certain drugs (251-253, 281), the use of these drugs in clinical practice may lead to secondary cancers characterized by MMR defects. In light of these problems, how can cancer chemotherapy be improved? Several strategies are apparent. First, since restoration of MMR to MMR-deficient tumor cells by gene transfer also restores their sensitivity to drugs (152, 153, 155), an improved therapy for MMR-deficient cancer could potentially include treatments that restore the tumors' MMR function, e.g., by gene or chromosomal transfer technology, prior to drug application. Thus, the MMR-restored tumor should be sensitive to regular chemotherapy. In addition, the development of drugs that could specifically kill MMR-deficient cells but not MMR-proficient cells could lead to progress in future cancer chemotherapy.

The genomic maintenance capability of MMR by both its repair and apoptotic functions underscores the importance of the MMR pathway in cancer biology and molecular medicine. Despite rapid advances in the field of MMR in the past decade, the molecular mechanisms by which the MMR pathway conducts mismatch correction and mediates DNA damage-induced apoptosis are still not fully understood. For example, how eukaryotic MMR determines stand-specificity and how MMR proteins interact with signal transducers to activate apoptotic machinery remain unknown. Understanding these questions will open new strategies for cancer-targeted drug design.

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Abbreviations: MMR, DNA mismatch repair; HNPCC, hereditary non-polyposis colorectal cancer; BER, base excision repair; NER, nucleotide excision repair; SSB, single-stranded DNA binding protein; ID, insertion/deletion; PCNA, proliferating cellular nuclear antigen; RPA, replication protein A; MSI, microsatellite instability; MSS, microsatellite stable; MSI-L, low microsatellite instability; MSI-H, high microsatellite instability; MNNG, *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine; 6-TG, 6-thioguanine; MTX, methotrexate; DHFR, dihydrofolate reductase; and nt, nucleotides.

Key words: Mismatch repair, HNPCC, Microsatellite instability, Cancer, Apoptosis, Review

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