Cell Research (2008) 18:85-98.

www.nature.com/cr

REVIEW

Mechanisms and functions of DNA mismatch repair

Guo-Min Li[1]

[1]*Graduate Center for Toxicology and Markey Cancer Center, Department of Molecular and Cellular Biochemistry, University of Kentucky College of Medicine, Lexington, KY 40536, USA*

DNA mismatch repair (MMR) is a highly conserved biological pathway that plays a key role in maintaining genomic stability. The specificity of MMR is primarily for base-base mismatches and insertion/deletion mispairs generated during DNA replication and recombination. MMR also suppresses homeologous recombination and was recently shown to play a role in DNA damage signaling in eukaryotic cells. *Escherichia coli* MutS and MutL and their eukaryotic homologs, MutSα and MutLα, respectively, are key players in MMR-associated genome maintenance. Many other protein components that participate in various DNA metabolic pathways, such as PCNA and RPA, are also essential for MMR. Defects in MMR are associated with genome-wide instability, predisposition to certain types of cancer including hereditary non-polyposis colorectal cancer, resistance to certain chemotherapeutic agents, and abnormalities in meiosis and sterility in mammalian systems.

***Keywords*:** MutS, MutL, microsatellite instability, cancer

Cell Research (2008) **18**:85-98. doi: 10.1038/cr.2007.115; published online 24 December 2007

Introduction

DNA damage accumulates in cells over time as a result of exposure to exogenous chemicals and physical agents (i.e., benzo[*a*]pyrene, polychlorinated biphenyls, dioxin, cigarette smoke, asbestos, ultraviolet light, radon), as well as endogenous reactive metabolites including reactive oxygen and nitrogen species (ROS and NOS). Another source of DNA damage is errors that occur during normal DNA metabolism or aberrant DNA processing reactions, including DNA replication, recombination, and repair. Nucleotide misincorporation generates DNA base-base mismatches during DNA synthesis at variable rates, depending on many factors, including the specific DNA polymerases. In general, the replicative DNA polymerases have relatively high replication fidelity (see McCulloch and Kunkel, this issue), while translesion DNA polymerases, which specifically bypass sites of DNA damage, have lower replication fidelity (see Andersen *et al*. and Gan *et al*. in this issue). DNA damage, if unrepaired, has the potential to generate mutations in somatic or germline cells, which can alter cellular phenotype and cause dysfunction and disease. To prevent such deleterious effects and safeguard the integrity of the genome, cells possess multiple mechanisms to repair DNA damage and thus prevent mutations. One such system is the critical pathway known as DNA mismatch repair (MMR).

MMR corrects DNA mismatches generated during DNA replication, thereby preventing mutations from becoming permanent in dividing cells [1-3]. Because MMR reduces the number of replication-associated errors, defects in MMR increase the spontaneous mutation rate [4]. Inactivation of MMR in human cells is associated with hereditary and sporadic human cancers [1, 3, 5], and the MMR system is required for cell cycle arrest and/or programmed cell death in response to certain types of DNA damage [6, 7]. Thus, MMR plays a role in the DNA damage response pathway that eliminates severely damaged cells and prevents both mutagenesis in the short term and tumorigenesis in the long term.

The prototypical *Escherichia coli* MMR pathway has been extensively studied and is well characterized both biochemically and genetically. Thus, *E. coli* MMR is a useful and important framework for understanding eukaryotic MMR. In this review, the biochemistry and genetics of *E. coli* MMR will be described briefly by way of introduction, and the remainder of the discussion will focus on the cellular functions of MMR and their roles in cancer avoidance in mammalian cells. For areas of research on human MMR not discussed in this paper or an additional discussion of MMR in other species, readers are referred to the following

Correspondence: Guo-Min Li
Tel: +1-859-257-7053; Fax: +1-859-323-1059
E-mail: gmli@email.uky.edu

excellent reviews: [1-3, 8-11].

Mechanism of mismatch correction

DNA MMR in E. coli

E. coli MMR requires the following protein components: 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 [12, 13]. MutS, MutL, and MutH initiate MMR and play specialized biological roles in MMR in *E. coli*.

MutS recognizes base-base mismatches and small nucleotide insertion/deletion (ID) mispairs, and thus MutS has been called the "mismatch recognition" protein [3]. MutS possesses intrinsic ATPase activity. High-resolution structures of MutS bound to DNA have been determined by X-ray crystallography [14, 15]. These structures revealed that MutS binds to a mismatch as a homodimer. Interestingly, the mismatch-binding site is comprised of sequence-wise identical but structurally and functionally different domains from the two subunits, indicating asymmetry in the protein-DNA complex. Hence, the MutS homodimer acts as a virtual heterodimer when bound to a DNA mismatch. This characteristic is mimicked by eukaryotic MutS homologs (MSH), which function as heterodimers instead of homodimers (see below). MMR in *E. coli* is ATP-dependent, and requires the functional MutS ATPase.

MutL interacts physically with MutS, enhances mismatch recognition, and recruits and activates MutH. Defects in MutL completely inhibit MMR in *E. coli*. Despite the fact that a functional human MutL homolog, MutLα, possesses an endonuclease activity that is essential for mammalian MMR [16], no hydrolytic activity has been detected in MutL. However, MutL may play a role as a molecular matchmaker that facilitates assembly of a functional MMR complex [3, 17], because it stimulates the loading and the processivity of helicase II (or UvrD) at the MMR initiation site [18, 19]. Like MutS, MutL functions as a homodimer and possesses ATPase activity [20]. Mutations in the ATP-binding domain lead to a dominant negative mutator phenotype [21]. MutL mutants 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 the activation of MutH by MutS [22]. Recent studies show that MutL interacts physically with the clamp loader subunits of DNA polymerase III [23, 24], suggesting that MutL may promote binding of DNA polymerase III to MMR intermediates. These observations suggest that MMR is coupled with DNA replication.

In *E. coli*, DNA is methylated at the N6 position of adenine in dGATC sequences. In replicating DNA, the daughter strand is transiently unmethylated, and it is the presence of hemimethylated dGATC sequences that molecularly distinguishes the newly synthesized daughter strand from the parental DNA strand. In MMR, hemimethylated dGATC sites determine the strand specificity of repair. MutH, which recognizes hemimethylated dGATC sequences, functions as a monomer and belongs to a family of type-II restriction endonucleases [25, 26]. Upon its recruitment and activation by MutS and MutL in the presence of ATP, MutH specifically incises the unmethylated daughter strand of hemimethylated dGATC [3, 22], and this strand-specific nick provides the initiation site for mismatch-provoked excision.

The first step of the MMR pathway is binding of a MutS homodimer to the mismatch. Subsequently, a hemi-methylated dGATC site 5′ or 3′ to the mismatch is located and cleaved by the concerted action of MutS, MutL, MutH, and ATP. Three models have been proposed to address how mismatch binding by MutS leads to cleavage of the hemimethylated dGATC site (see the section of Unsolved Fundamental Problems in MMR for details). The strand-specific nick generated by MutH at hemimethylated dGATC is a starting point for excision of the mispaired base. In the presence of MutL, helicase II (UvrD) loads at the nick and unwinds the duplex from the nick towards the mismatch [18], generating single-strand DNA, which is rapidly bound by single-stranded DNA-binding protein (SSB) and protected from nuclease attack [27]. Depending on the position of the strand break relative to the mismatch, ExoI or ExoX (3′→5′ exonuclease), or ExoVII or RecJ (5′→3′ exonuclease) excises the nicked strand from the nicked site (the dGATC site) up to and slightly past the mismatch. The resulting single-stranded gap undergoes repair DNA resynthesis and ligation by DNA polymerase III holoenzyme, SSB, and DNA ligase [3].

These early studies on *E. coli* MMR demonstrate three key features of this important pathway: first, repair is strand specific (i.e., restricted to the newly synthesized DNA strand); second, repair is bi-directional, proceeding 5′→3′ or 3′→5′ from the nick to the site of the mismatch; and third, MMR has broad substrate specificity including base-base mismatches and small ID mispairs. All of these properties require functional MutS, MutL, and MutH. Because the mechanism of MMR is highly conserved throughout evolution, *E. coli* MMR is an excellent model for MMR in eukaryotic cells.

MMR in human cells

MMR is a highly conserved biological pathway with strong similarities between human MMR and prototypical *E. coli* MMR [2, 3]. These similarities include substrate

specificity, bidirectionality, and nick-directed strand specificity. The role of hemi-methylated dGATC sites as a signal for strand discrimination is not conserved from *E. coli* MMR to human MMR, but because the hemi-methylated dGATC site directs MutH-dependent nicking, and because human MMR is presumed to be nick-directed *in vivo*, both systems are thought to discriminate daughter and template strands using a strand-specific nick.

Several human MMR proteins have been identified based on their homology to *E. coli* MMR proteins (Table 1). These include human homologs of MutS [28-32], MutL [33-36], EXO1 [37-39], single-strand DNA-binding protein RPA [27, 40], proliferating cellular nuclear antigen (PCNA) [41-43], DNA polymerase δ (pol δ) [44], and DNA ligase I [45]. Although *E. coli* MutS and MutL proteins are homodimers, human MutS and MutL homologues are heterodimers [32, 34, 46]. hMSH2 heterodimerizes with hMSH6 or hMSH3 to form hMutSα or hMutSβ, respectively, both of which are ATPases that play a critical role in mismatch recognition and initiation of repair [2]. hMutSα preferentially recognizes base-base mismatches and ID mispairs of 1 or 2 nucleotides, while hMutSβ preferentially recognizes larger ID mispairs. At least 4 human MutL homologs (hMLH1, hMLH3, hPMS1, and hPMS2) have been identified [33, 35, 36, 47]. hMLH1 heterodimerizes with hPMS2, hPMS1, or hMLH3 to form hMutLα, hMutLβ, or hMutLγ, respectively [2]. hMutLα is required for MMR and hMutLγ plays a role in meiosis, but no specific biological role has been identified for hMutLβ [2]. hMutLα possesses an ATPase activity and defects in this activity inactivate MMR in human cells. In a reconstituted human MMR system, hMutLα regulates termination of mismatch-provoked excision [45]. Recent studies show that MutLα possesses a PCNA/replication factor C (RFC)-dependent endonuclease activity which plays a critical role in 3′ nick-directed MMR involving EXO1 [16].

PCNA interacts with MSH2 and MLH1 and is thought to play roles in the initiation and DNA resynthesis steps of MMR [41, 43]. PCNA also interacts with MSH6 and MSH3 [48-51] via a conserved PCNA interaction motif termed the PIP box [52]. It has been proposed that PCNA may help localize MutSα and MutSβ to mispairs in newly replicated DNA [53, 54]. Although PCNA is absolutely required during 3′ nick-directed MMR, it is not essential during 5′ nick-directed MMR [55]. This observation might be explained by the fact that EXO1, a 5′→3′ exonuclease, is involved in both 5′ and 3′ directed MMR. Like PCNA, EXO1 also interacts with MSH2 and MLH1 [37-39, 56-58]. While EXO1 can readily carry out 5′ directed mismatch excision in the presence of MutSα or MutSβ and RPA [45, 59], its role in catalyzing 3′ nick-directed excision requires the MutLα endonuclease, which is activated by PCNA and RFC [16, 60]. Although it has been suggested that EXO1 possesses a cryptic 3′→5′ exonuclease activity [60, 61], current data do not support that hypothesis. Instead, recent studies suggest the following steps for EXO1-catalyzed 3′

Table 1 MMR components and their functions

E. coli	Human	Function
$(MutS)_2$	hMutSα (MSH2-MSH6)[a]	DNA mismatch/damage recognition
	hMutSβ (MSH2-MSH3)	
$(MutL)_2$	hMutLα (MLH1-PMS2)[a]	Molecular matchmaker; endonuclease, termination of mismatch-provoked excision
	hMutLβ (MLH1-PMS1)	
	hMutLγ (MLH1-MLH3)	
MutH	?[b]	Strand discrimination
UvrD	?[b]	DNA helicase
ExoI, ExoVII, ExoX, RecJ	ExoI	DNA excision; mismatch excision
Pol III holoenzyme	Pol δ	DNA re-synthesis
	PCNA	Initiation of MMR, DNA re-synthesis
SSB	RPA	ssDNA binding/protection; stimulating mismatch excision; termination of DNA excision; promoting DNA resynthesis
	HMGB1	Mismatch-provoked excision
	RFC	PCNA loading; 3' nick-directed repair; activation of MutLα endonuclease
DNA Ligase	DNA ligase I	Nick ligation

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

nick-directed repair: (1) after recognition of the 3′ nick and the mismatch, MutLα endonuclease makes an incision 5′ to the mismatch in a manner dependent on PCNA and RFC; and (2) EXO1 performs 5′→3′ excision from the MutLα-incision site through and beyond the site of the mismatch [16]. However, *exo1* null mutants in mice and yeast have a weak mutator phenotype [56, 62]; thus, it is likely that additional as yet unidentified exonucleases are involved in eukaryotic MMR.

Other protein components involved in human MMR include single-strand DNA-binding protein RPA, RFC, high mobility group box 1 protein (HMGB1), and DNA pol δ. RPA seems to be involved in all stages of MMR: it binds to nicked heteroduplex DNA before MutSα and MutLα, stimulates mismatch-provoked excision, protects the ssDNA gapped region generated during excision, and facilitates DNA resynthesis [27, 45, 60, 63]. Furthermore, RPA is phosphorylated after pol δ is recruited to the gapped DNA substrate. Recent studies indicate that phosphorylation reduces the affinity of RPA for DNA, that unphosphorylated RPA stimulates mismatch-provoked DNA excision more efficiently than phosphorylated RPA, and that phosphorylated RPA facilitates MMR-associated DNA resynthesis more efficiently than unphosphorylated RPA [63]. These results are consistent with the fact that a high-affinity RPA-DNA complex might be required to protect nascent ssDNA and to displace DNA-bound MutSα/MutLα [27, 45], while a lower-affinity RPA-DNA complex might facilitate DNA resynthesis by pol δ [63]. HMGB1 is a mismatch-binding protein and has a DNA-unwinding activity [64-66]. It interacts with MSH2 and MSH6 *in vitro* [67]. Recent studies show that HMGB1 can substitute for RPA in an *in vitro* reconstituted MMR system [45]. Additional studies are needed to precisely define the function of HMGB1 in MMR.

Unsolved fundamental problems in MMR

Despite great progress in identifying MMR proteins and genes and application of state-of-the-art biochemical and genetic approaches to analyze the mechanism of MMR in prokaryotic and eukaryotic cells, several key questions about this pathway remain unanswered. One of these questions concerns the mechanism by which MMR proteins facilitate the communication between two physically distant DNA sites: the mismatch and the strand discrimination signal. It is generally agreed that the strand discrimination signal is a strand-specific nick in both prokaryotic and eukaryotic cells (see above), although the source of the nicking activity, at least for the leading strand, is not known in eukaryotic cells. Previous studies have proposed several alternative models for this process, which can be classified into "*cis*-" or "moving" and "*trans*-" or "stationary" models (Figure 1). The "stationary" model (Figure 1, right) proposes that interactions among MMR proteins induce DNA bending or looping that brings the two distant sites together, while MutS (or the MSH heterodimers, i.e. MutSα and MutSβ) remains bound at the mismatch [19, 22]. In this model, the MutS (or MSH heterodimers) ATPase activity acts in a proofreading role to verify mismatch binding and authorize the downstream excision [22]. Support for the stationary model came from the following experiments. Junop *et al*. showed that recognition of a mismatch by MutS on a DNA molecule activated MutH cleavage of a GATC site located on a separate DNA molecule without a mismatch [22]. Consistent with this observation, a second study demonstrated that mismatch-provoked excision could be initiated when a biotin-streptavidin blockade was placed between the mismatch and pre-existing nick [68]. The "*cis*" or "moving" models suggest that MutS-MutL (or MutSα/β-MutLα) complexes load at a mismatch site and then move away from the site to search for the strand break, where exonucleases can be recruited to initiate excision.

There are two moving models, one called the "translocation" model and the other called the "molecular switch" or "sliding clamp" model (Figure 1). In the translocation model [69], ATP reduces the mismatch-binding affinity of MutS or the MSH heterodimers, and ATP hydrolysis drives unidirectional translocation of MutS proteins along the DNA helix. DNA is threaded through the protein complex until the latter reaches a strand discrimination signal in either orientation, a process that forms a DNA loop (Figure 1, left). In the molecular switch model, MutS or the MSH heterodimer binds to mismatched DNA in an ADP-bound state. The mismatch binding by MutS or the MSH heterodimer triggers a conformational change that allows an ADP to ATP exchange, which promotes a second conformational change that allows MutS or the MSH heterodimers to form a sliding clamp [70-73]. In this model (Figure 1, middle), it is the binding of ATP, not ATP hydrolysis, that signals downstream events including formation of ternary complex with MutL (or MLH heterodimers) and sliding of the ternary complex from the mismatch to the strand break [70-73].

Recent studies by Pluciennik and Modrich [74] argue in favor of a moving rather than a stationary mechanism, because their data demonstrate that a dsDNA break [75] or a protein "roadblock" between the mismatch and the nick inhibits *in vitro* MMR with recombinant *E. coli* proteins. It is not clear why two "roadblock" experiments [68, 74] obtained distinct results. In a reconstituted human MMR reaction, Zhang *et al*. [45] show that multiple MutSα-MutLα complexes are essential for processing a single mismatch, providing evidence to support the molecular switch model. Additional studies are needed to address these unresolved

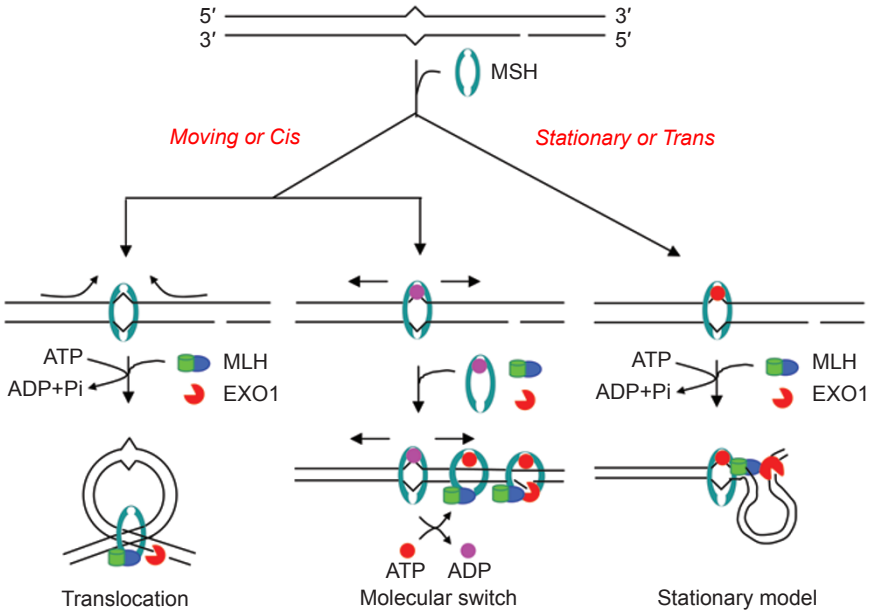

Figure 1 Models for signaling downstream MMR events following mismatch recognition. A schematic diagram for signaling between the mismatch and the strand discrimination signal is shown. Here, a 5′ nick is the strand discrimination signal. Similar models apply for 3′ nick-directed MMR. The "stationary" or "*trans*" model (right) emphasizes that MutS or its homolog (MSH) proteins remain bound at the mismatch. It is the protein-protein interactions that induce DNA bending or looping that brings the two distant sites together. The two DNA sites can cooperate in a "*trans*" configuration. In two "*cis*" or "moving" models, one called the "translocation" model (left) and the other called the "molecular switch" or "sliding clamp" model (middle), it is hypothesized that the MSH proteins bind to the mismatch and then move away from the site to search for the strand discrimination signal. The translocation model suggests that ATP hydrolysis drives unidirectional movement of the MSH proteins, resulting in the formation of an α-like loop. In the molecular switch model (center), binding of an MSH protein (in its ADP-bound state) to the mismatch triggers an ADP to ATP exchange that promotes bi-directional sliding of the protein away from the mismatch, thereby emptying the mismatch site for an incoming MSH protein. Mismatch excision begins when an MSH protein reaches the strand break.

questions about the molecular mechanism of MMR.

MMR mediates DNA damage signaling

MMR deficiency and drug resistance

DNA-damaging agents such as the alkylating agents *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), temozolomide, or procarbazine are cytotoxic agents that kill most of the replicating cells. Many cancer therapeutics are genotoxic and cytotoxic agents that induce apoptotic cell death. Interestingly, many cells that acquire resistance to such agents are deficient in MMR. For example, the human lymphoblastoid cell line MTI, which has a defect in *hMSH6*, was derived by culturing TK6 cells in the presence of a high concentration of MNNG. The resulting MNNG-resistant MT1 cells are defective in strand-specific MMR [76]. Many human colorectal cancer cell lines are also resistant to alkylating agents and have associated defects in MMR. The causal relationship between drug resistance and MMR is demonstrated by the fact that *hMLH1*-defective MNNG-resistant cells lose drug resistance when the *hMLH1* defect is genetically complemented with wild-type *hMLH1* on chromosome 3 [77]. It has also been observed that defects in *MSH2* and *PMS2* confer resistance to alkylating agents (reviewed in [78]). The mechanism by which MMR influences drug cytotoxicity is discussed further below.

Resistance to methotrexate (MTX) has also been associated with phenotypic changes in MMR in human cells. This occurs by the unusual mechanism of co-amplification of the human chromosomal region that encodes dihydrofolate reductase (DHFR, the target of MTX) and *hMSH3* [79, 80]. Amplification of *DHFR* lowers sensitivity to MTX by overexpressing the target of the drug. However, overexpression of *hMSH3* sequesters hMSH2 in the hMutSβ heterodimer, effectively preventing formation of the hMutSα (hMSH2/hMSH6) heterodimer, which leads to degradation of uncomplexed hMSH6, significant dysregulation of MMR and hypermutability [81, 82]. Overexpression of DHFR combined with genome-wide hypermutability and defective MMR are likely responsible for the MTX resistance of HL60 and other tumor cells.

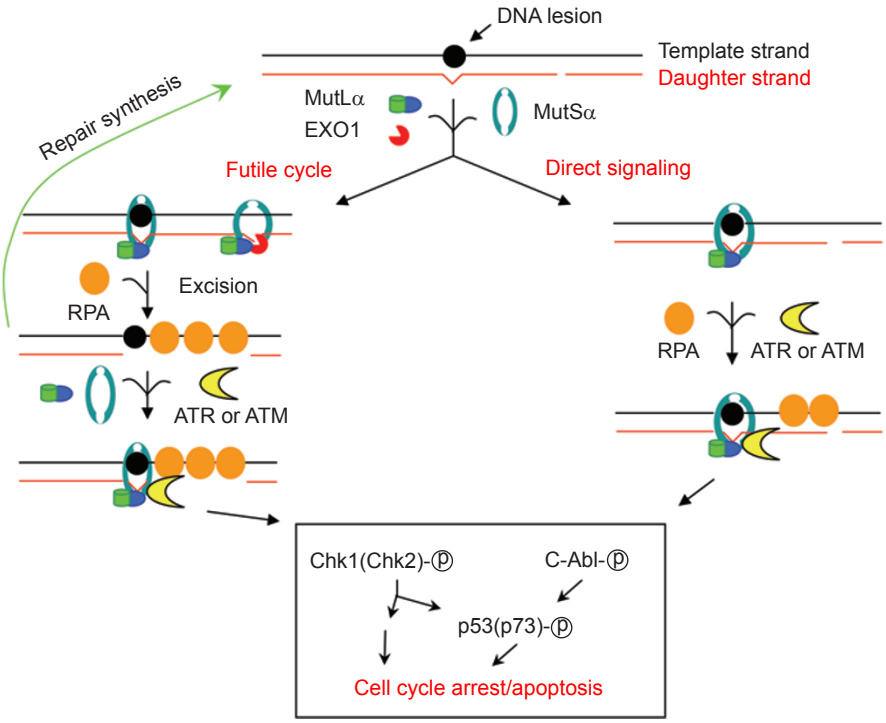

Figure 2 Models for MMR-dependent DNA damage signaling. The "futile DNA repair cycle" model (left) suggests that DNA adducts (solid black circle) induce misincorporation, which triggers the strand-specific MMR reaction. Since MMR only targets the newly synthesized strand for repair, the offending adduct in the template strand cannot be removed, and will provoke a new cycle of MMR upon repair resynthesis. Such a futile repair cycle persists and activates the ATR and/or ATM damage signaling network to promote cell cycle arrest and/or programmed cell death. The direct signaling model proposes that recognition of DNA adducts by MSH-MLH complexes allows the proteins to recruit ATR and/or ATM to the site, activating the downstream damage signaling.

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

Cell cycle arrest is an important mechanism for preventing DNA damage-induced genomic instability. A large number of studies have characterized the so-called G2 or S phase checkpoints, and identified proteins required for cell cycle arrest, including ATM, ATR, p53, p73, Chk1, and Chk2. However, it was a somewhat unexpected finding that hMutSα- and hMutLα-deficient cells are defective in cell cycle arrest in response to multiple types of DNA damaging agents [6, 7, 83]. While the molecular basis of this effect is not precisely known, it has been reported that MMR-deficient cells fail to phosphorylate p53 and p73 in response to DNA damage [84, 85]. This implicates ATM, ATR, and/or c-Abl, because these kinases phosphorylate p53 and p73 during the response to DNA damage [85, 86]. In support of this, it has been reported that hMutSα and hMutLα interact physically with ATM, ATR-ARTIP, c-Abl, and p73 in cells treated with DNA damaging agents/drugs [83, 87-89]. These observations implicate hMutSα and hMutLα in a signaling cascade that leads from DNA damage to cell cycle arrest and/or apoptosis. They also at least in part explain the fact that drug-induced cytotoxicity is lost in MMR-deficient cells, as discussed above [6]. Very recently, EXO1 has been shown to be essential for upstream induction of DNA damage response, possibly by reducing ssDNA formation and recruiting RPA and ATR to the damage site [90]. It remains to be seen if MutSα and/or MutLα act to recruit EXO1 in DNA damage response as they do in MMR.

Two models have been proposed to describe the role of MMR in DNA damage signaling. The "futile DNA repair cycle" model (Figure 2, left) proposes that strand-specific MMR, which targets only newly replicated DNA, engages in a futile DNA repair cycle when it encounters DNA lesions in the template strand, and this futile cycling activates DNA damage signaling pathways to induce cell cycle arrest and apoptosis [6]. Support for this model came from both *in vivo* and *in vitro* experiments. Stojic *et al*. [86] showed that exposure to MNNG induces DNA breaks/gaps, cell cycle arrest, and persistent nuclear foci at sites of DNA damage. The DNA damage-associated repair foci contain both damage signaling and DNA repair proteins, including ATR, γ-H2AX, and RPA. York and Modrich [91] showed that nicked circular heteroduplex plasmid DNA containing a single O^6-methylguanine (O^6-me-G)-thymine (T) mispair cannot be repaired by the MMR system when the lesion (O^6-me-G) and the nick are on opposite strands; this suggests a futile repair process. An alternative model, referred to as the direct signaling model (Figure 2, right), argues that hMutSα/hMutLα directly trigger DNA damage signaling by recruiting ATM or ATR/ARTIP to the lesion, which activates a checkpoint response. This model is supported by an elegant study from the Hsieh laboratory showing that ATR and ATRIP form a complex with MutSα/MutLα in the presence of O^6-me-G/T, which activates the ATR kinase

and phosphorylates Chk1 [89]. Because mammalian MMR proteins interact with a broad spectrum of DNA lesions [6], this model is consistent with the notion that MutSα/MutLα acts as a sensor for DNA damage in mammalian cells. Both models provide a reasonable explanation for decreased DNA damage-induced apoptotic signaling and increased drug resistance in MMR-deficient cells.

Role of MMR in other DNA metabolic pathways

MMR proteins have also been implicated in homeologous recombination, immunoglobulin class switching, somatic hypermutation, interstrand-crosslink repair, and trinucleotide repeat (TNR) expansion. Homeologous recombination (recombination between related but non-identical DNA sequences) generates mispairs/heteroduplexes, and induces genomic instability via chromosomal translocations, deletions, or inversions [92, 93]. The frequency of homeologous recombination is much lower than that of homologous recombination in normal cells, but the frequency of homeologous recombination is dramatically elevated in MMR-deficient cells, suggesting that MMR suppresses homeologous recombination [3, 94]. MutS and MutL inhibit DNA strand exchange between divergent sequences *in vitro*, most likely by binding to the mismatches generated during strand exchange [95]. Recent studies in yeast reveal that suppression of homeologous recombination is mediated by MutSα and a RecQ family helicase, SGS1 [96, 97]. Consistent with this notion, yeast strains defective in *sgs1* fail to suppress homeologous recombination [97, 98]. It has been postulated that MutSα recruits SGS1 to DNA mismatches, where it unwinds the heteroduplex and blocks homeologous recombination [96]. Although suppression of homeologous recombination by MMR proteins in human cells is less well understood, two human SGS1 homologous proteins, BLM and RECQ1, interact with MutSα [99, 100]. This suggests that a similar mechanism is used to suppress homeologous recombination in yeast and human cells.

Recent studies also implicate MMR in repair of inter-strand crosslinks (ICLs), in a process that involves protein components from homologous recombination, double-strand break repair, and nucleotide excision repair [101-103]. The precise nature of this involvement is not yet clear, and the specific MMR proteins that participate remain somewhat controversial. However, hMutSβ appears to directly bind ICLs *in vitro* [102], and hMutLα interacts specifically with the helicase domain of Fanconi Anemia protein FANC-J to facilitate ICL repair [101].

The studies discussed above suggest that MMR promotes genomic stability. However, during immunoglobulin class switching and somatic hypermutation, it appears that MMR proteins play a highly specialized role in promoting genetic variation. Immunoglobulin class switching and somatic hypermutation are mechanisms for increasing antibody diversity during antigen-stimulated B-cell differentiation. During this process, activation-induced cytidine deaminase (AID) deaminates cytosine residues to uracil, generating G:U mispairs, which can be recognized and processed by MMR [104, 105]. However, during repair resynthesis, the high-fidelity replicative pol δ and ε are thought to be replaced by the translesion polymerases, which are error-prone and crucially introduce base substitutions and frameshift mutations [106]. Additionally, MMR proteins play an important role in class switch recombination, an event where the IgM constant region is substituted by downstream constant sequences. In this capacity, MMR proteins utilize strand breaks generated by uracil DNA glycosylase to repair the AID-induced G:U mispairs in a strand-indiscriminate manner, leading to double-strand DNA breaks. It is these breaks that stimulate class switch recombination [107]. B cells from mice deficient in MMR genes (*MSH2, MSH6, MLH1, PMS2,* or *EXO1*) display a low level of somatic hypermutation and reduced class switch recombination [8, 62, 108, 109].

MMR proteins also promote TNR expansion, a phenomenon associated with a number of neurological disorders in humans, including Huntington's disease, myotonic dystrophy, and fragile X syndrome [110] (also see Kovtun and McMurray in this issue). TNRs such as $(CAG)_n$ form single-stranded DNA loop/hairpin structures *in vitro* [111]. Surprisingly, transgenic CAG repeats undergo expansion in wild-type, but not in knockout mice defective in MMR genes *MSH2* and *MSH3* [112, 113], suggesting that the expansion mutations of the transgenic CAG repeats require functional MMR proteins MSH2 and MSH3. *In vitro* biochemical studies indeed show that MutSβ (the MSH2-MSH3 heterodimer) specifically binds to the $(CAG)_n$ hairpin structure [113]. One model proposes that MutSβ inhibits repair or resolution of the $(CAG)_n$ hairpin, thus stimulating $(CAG)_n$ expansion [113]. However, many of these studies were conducted using transgenic mouse models for TNR expansion. Thus, the role of MMR in human neurological diseases involving TNR expansion is at present unclear, and additional genetic and biochemical studies are needed to define the mechanism of TNR expansion in human cells.

MMR deficiency leads to cancer development

MMR defects in hereditary non-polyposis colorectal cancer (HNPCC) and other cancers

In the early 1990s, it was shown that HNPCC and some cases of sporadic colon cancer are caused by defects in human MMR [3]. For HNPCC, Kolodner and co-workers,

and Vogelstein and co-workers independently identified germ-line mutations in *hMSH2* at chromosome 2p16-p21 in HNPCC families [29, 30]. Genetic analyses of HNPCC kindreds revealed a large increase in frequency of insertion and deletion mutations in simple repeat (microsatellite) sequences, a phenomenon known as microsatellite instability (MSI) [114]. MSI was also observed at lower incidence in sporadic colon cancers [114-116]. Additional studies showed 100- to 700-fold decreased stability of poly(GT) tracts in yeast strains with single or double knockouts in *MSH2*, *MLH1*, or *PMS1* [117]. Biochemical studies by Modrich and co-workers [118] and Kunkel and co-workers [119] also showed that extracts of MSI-positive tumor cells were severely defective in repair of base-base and ID mispairs. Thus, genetic and biochemical evidence converged to support the hypothesis that defective MMR plays a causal role in carcinogenesis leading to HNPCC, and strongly implicated such defects in some sporadic human cancers such as colorectal cancer.

The second locus linked to HNPCC was *hMLH1* at 3p21-p23 [33, 36, 120]. Furthermore, HNPCC has also been linked to mutations in two additional MutL homolog genes *hPMS1* and *hPMS2*, on 2q and 7p, respectively [35]. Defects in *hMLH1* represent the majority of all HNPCC cases [35, 36], with mutations in *hMSH2* accounting for a large fraction of all the remaining HNPCC cases for which a genetic defect has been identified. In contrast, germ-line mutations in *hMSH3* have not yet been linked to HNPCC, and mutations in *hPMS1*, *hPMS2*, and *hMSH6* are relatively rare in HNPCC patients [1-3]. These observations are consistent with the fact that *hMSH2* and *hMLH1* are essential MMR components, while *hPMS1*, *hPMS2*, *hMSH6*, and *hMSH3* play important but partially redundant and/or dispensable roles in MMR.

Genetic evidence described above shows that defects in MMR correlate with HNPCC, and biochemical studies provide compelling additional evidence for this hypothesis. In particular, cell lines from HNPCC patients and sporadic tumors with MSI are defective in strand-specific MMR, and these cells can be divided into at least two complementation groups, corresponding to hMutLα [34] and hMutSα [32]. Importantly, purified hMutLα or hMutSα specifically complements the biochemical defect in these cells. Similar evidence was obtained from chromosome or gene transfer experiments: expressions of the exogenous MMR genes complement the biochemical defect and stabilize simple repetitive sequences in the transfected cells (reviewed in [121]).

These genetic and biochemical complementation studies essentially prove the causal role of MMR defects in HNPCC. However, many questions remain, including whether or not mutations in *hMLH3* or *hEXOI* also cause HNPCC, a possibility that remains controversial [47, 122, 123]. Mice defective in *EXOI* exhibit a mild defect in genomic stability, are partially defective in strand-specific MMR, and have increased rates of some cancers [62]. Thus, it seems possible that germ-line mutations in *hEXOI* could potentially have similar phenotypic effects in humans. Additional studies are needed to clarify this point.

Although MSI was first correlated with MMR defects in tumors from HNPCC patients, MSI is also associated with a wide variety of non-HNPCC and non-colonic tumors (reviewed in Ref. [124]). These include endometrial, ovarian, gastric, cervical, breast, skin, lung, prostate, and bladder tumors as well as glioma, leukemia, and lymphoma. Biochemical studies confirmed that MSI cell lines from sporadic leukemia, endometrial, ovarian, prostate, and bladder cancers are defective in strand-specific MMR [32, 125, 126]. Interestingly, MSI in sporadic non-colonic tumors is often associated with hypermethylation of the promoter of *hMLH1* (see below for details), and few mutations in MMR genes have been identified in these cells. These findings suggest that MMR defects are a likely cause of MSI in non-colonic sporadic cancers, although other mechanisms may also be involved in causing the MSI mutator phenotype.

Mouse models for MMR demonstrate roles in cancer and meiosis

Knockout mouse models have been developed for *MSH2*, *MSH3, MSH6, MLH1*, *MLH3, PMS1*, *PMS2*, and *EXO1* (reviewed in [8, 62, 127, 128]) and their phenotypes have been somewhat informative. Most of the knockout mice have a mutator phenotype, are MSI-positive, and are cancer-prone. However, the primary cancer susceptibility of *MSH2*, *MLH1*, and *PMS2* knockout mice is lymphoma, not colorectal cancer as in humans, and secondary cancer susceptibilities are to gastrointestinal tumors, skin neoplasms, and/or sarcomas (Table 2).

MSH2$^{-/-}$ deficient mice are fertile [129, 130], are MSI-positive, develop lymphoma within 1 year of age, and have a significantly shorter lifespan than wild-type mice (i.e., 50% mortality by 6 months of age). The phenotype of *MSH6*$^{-/-}$ deficient mice is similar to that of *MSH2*$^{-/-}$ deficient mice, but lacking MSI [129, 131], a phenotype resembling that of atypical HNPCC with an *hMSH6* defect as the tumors in these *MSH6*-defective individuals have longer latency and low MSI [132]. Cells from *MSH3*$^{-/-}$ mice are defective in repair of ID mispairs but can repair base-base mismatches. *MSH3*$^{-/-}$ mice are either tumor free [133] or develop tumors at a very late age [134], essentially consistent with the fact that no *MSH3* mutations have been identified in HNPCC patients. However, in *MSH3*$^{-/-}$ *and* *MSH6*$^{-/-}$ double deficient mice, the tumor predisposition phenotype is indistinguishable from *MSH2*$^{-/-}$ or *MLH1*$^{-/-}$

mice [133, 134], suggesting that *MSH3* cooperates with *MSH6* in tumor suppression.

Sterility is a characteristic feature of *MLH* mutant mice (except *PMS1*) [135-137]. These animals are also susceptible to cancer and display genomic instability, reflecting defective MMR. However, male and female *MLH1* and *MLH3* knockout mice [135-137], and male *PMS2* knockout mice are completely sterile [138]. *PMS1* knockout mice are exceptional, because they are fertile, they lack cancer susceptibility, and, apart from a very small increase in mutations in mononucleotide repeats, they appear to be MSI-negative [127]. *EXO1* defective mice are also sterile [62]. It is clear that the loss of fertility in these knockout mice is caused by abnormal meiosis [62, 128, 135-137].

The characteristics of all MMR knockout mouse models are summarized in Table 2. These data strongly support the ideas that MMR is a basic genome surveillance mechanism and that defects in MMR can promote cancer development. The effects of MMR defects on carcinogenesis appear to be tissue- and species-specific, in a manner that is poorly understood. The effects of MMR defects on meiosis in humans remain poorly characterized. However, MMR clearly plays a critical role during meiosis and/or gamete formation in mice.

Epigenetic silencing of MMR gene expression leads to cancers

Mutations in MMR genes cause genomic instability and MSI in HNPCC and in a subset of sporadic colorectal cancers. However, in a significant fraction of MSI-positive sporadic colon tumors that have an MMR defect, mutations have not been identified in MMR genes. Epigenetic silencing of *hMLH1* via promoter hypermethylation strongly down-regulates MMR in many of these cases [139, 140]. In contrast, hypermethylation of the *hMSH2* gene is rarely observed in tumors with MSI. In fact, it has been reported that more than 95% of MSI-H sporadic tumors demonstrate mutation and/or epigenetic silencing of *hMLH1* [141]. While most studies demonstrate epigenetic silencing of the *hMLH1* promoter in sporadic tumors, hypermethylation of the *hMLH1* promoter was also recently demonstrated in an HNPCC patient who does not have a germ-line mutation in any MMR gene [142]. Interestingly, recent studies suggest that this effect may be heritable [143-145]. Direct evidence that *hMLH1* promoter hypermethylation down-regulates *hMLH1* gene expression was obtained by treating cells with 5-aza-deoxycytidine, which reversed promoter hypermethylation, and restored *hMLH1* gene expression and normal MMR capacity [146, 147].

MMR deficiency and mutations in coding repeat sequences

Previous studies demonstrate that defects in MMR increase the mutation rate in genes containing a simple repeat sequence in coding regions, often referred to as target genes [148]. Thus, defects in MMR confer a mutator phenotype. It is presumed that such a mutator phenotype has genome-wide consequences and could increase the frequency of additional genome-destabilizing and cancer-promoting mutations; however, this is a difficult hypothesis to test experimentally. One approach is to selectively analyze the stability of di- and tri-nucleotide tracts within coding regions. For example, Markowitz *et al.* [149] reported two mutation "hotspots" in the *type II transforming growth factor-β receptor* (*TGF-β RII*) gene in MMR-deficient tumor cells from a patient with sporadic colorectal cancer. One of these mutational hotspots fell within a 6-bp GT dinucleotide repeat and the other fell within an $(A)_{10}$ mononucleotide repeat [149]. Both of these hotspots were sites of frequent frameshift mutations that truncated the *TGF*-β *RII* gene product. Similar observations have been made in other colorectal tumor cells and many other MSI-positive tumor cells including glioma, gastric, uterine, cervical and squamous head and neck tumors, as well as ulcerative colitis-associated cancer and cecum cancer. Furthermore, in some of these tumor cells, somatic frameshift mutations have been documented in many other genes including *Bax*, *insulin-like growth factor 2 receptor* (*IGF2-R*), transcrip-

Table 2 Phenotypes of MMR-deficient knockout mice

Gene	MSI	Tumor	Fertility	Reference
MSH2	Yes	Lymphoma, GI, skin, and other tumors	Yes	[129, 130]
MSH3	Yes	Tumor free or GI tumors at a very late age	Yes	[133, 134]
MSH6	Low MSI in dinucleotide repeats	Lymphoma, GI and other tumors	Yes	[131, 133, 134]
MLH1	Yes	Lymphoma, GI, skin, and other tumors	No	[135, 136]
PMS1	Mononucleotide repeats only	None	Yes	[127]
PMS2	Yes	Lymphoma and sarcoma	Male infertile	[127, 138]
MLH3	Yes	Not available	No	[137]
EXO1	Yes	Lymphoma	No	[62]

tion factor *E2F-4*, *APC*, *PTEN*, *hMSH3*, *hMSH6*, *Mre11*, *MBD4/MED, ACTRII, AIM2, APAF-1, AXIN-2, BCL-10, BLM, Caspase-5, CDX-2, CHK-1, FAS, GRB-14,* cell cycle protein *hG4-1, KIAA0977,* ubiquinone oxidoreductase gene *NADH, OGT, Rad50, RHAMM, RIZ, SEC63, SLC23AT, TCF-4*, and *WISP-3* (reviewed in Ref. [150]). These data are consistent with the idea that similar mutations occur on a genome-wide basis and at a much higher rate in MMR-deficient cells than in wild-type cells. Because the genes noted above play critical roles in regulating cell growth or genomic stability, loss-of-function mutations in these genes may be crucial steps in the multi-step pathway of carcinogenesis.

Conclusion and perspectives

The discovery that defects in MMR play a causal role in HNPCC and many MSI-positive sporadic cancers brought immediate clinical relevance to research in the field of eukaryotic MMR. This discovery led to intensive research on and better understanding of the biological roles of MMR in eukaryotic cells, which relate to cancer prevention and therapy. Although the primary role of MMR is to improve replication fidelity by correcting replication-associated base-base mismatches or ID mispairs, important secondary roles are to modulate DNA recombination and facilitate DNA damage signaling. Thus, it is abundantly clear that defects in MMR are "permissive" for carcinogenesis.

The identification that MMR-deficient cells are resistant to certain chemotherapeutic drugs such as temozolomide, procarbazine, or cisplatin has significant impacts on cancer treatments, especially for patients with tumors defective in MMR. It is also known that MMR deficiency can be acquired during chemotherapy by selective mutations in MMR genes [6]. Therefore, the risks for chemotherapy are two-fold. First, for patients with MMR-deficient tumors, chemotherapeutic treatments could selectively kill patients' MMR-proficient cells (e.g., blood cells) that undergo proliferation, thereby leading to rapid deaths of the cancer patients. Second, if a patient's tumor is not caused by loss of MMR function, chemotherapeutic treatments may kill the tumor cells; at the same time, the treatment may induce or select for a mutation in MMR genes, which could lead to a secondary cancer. Therefore, novel chemotherapeutic or alternative approaches are needed for cancer patients with or without MSI-positive tumors. Such approaches might include targeted gene therapy, which could selectively restore drug sensitivity in tumor cells defective in MMR or treatment with agents that stimulate apoptosis downstream of MMR in tumor cells. Additional research on mechanisms that selectively kill MMR-deficient cells is also warranted. Such efforts should also include more basic research on the molecular mechanisms of eukaryotic MMR. Understanding these mechanisms will support efforts for developing new therapeutic approaches for patients with HNPCC or other MSI-positive MMR-deficient tumors.

Acknowledgments

The author acknowledges research support from the National Institutes of Health (GM072756 and CA115942) and the Kentucky Lung Cancer Research Program, USA. The author regrets the lack of citations for many important observations mentioned in the text, but their omission is made necessary by restrictions in the preparation of this review. The author holds the James-Gardner Endowed Chair in Cancer Research.

References

1 Kolodner RD, Marsischky GT. Eukaryotic DNA mismatch repair. *Curr Opin Genet Dev* 1999; **9**:89-96.
2 Kunkel TA, Erie DA. DNA mismatch repair. *Annu Rev Biochem* 2005; **74**:681-710.
3 Modrich P, Lahue R. Mismatch repair in replication fidelity, genetic recombination, and cancer biology. *Annu Rev Biochem* 1996; **65**:101-133.
4 Tiraby JG, Fox MS. Marker discrimination in transformation and mutation of pneumococcus. *Proc Natl Acad Sci USA* 1973; **70**:3541-3545.
5 Lynch HT, de la Chapelle A. Genetic susceptibility to non-polyposis colorectal cancer. *J Med Genet* 1999; **36**:801-818.
6 Li GM. The role of mismatch repair in DNA damage-induced apoptosis. *Oncol Res* 1999; **11**: 393-400.
7 Stojic L, Brun R, Jiricny J. Mismatch repair and DNA damage signalling. *DNA Repair (Amst)* 2004; **3**:1091-1101.
8 Buermeyer AB, Deschenes SM, Baker SM, Liskay RM. Mammalian DNA mismatch repair. *Annu Rev Genet* 1999; **33**:533-564.
9 Jiricny J. The multifaceted mismatch-repair system. *Nat Rev Mol Cell Biol* 2006; **7**:335-346.
10 Yang W. Structure and function of mismatch repair proteins. *Mutat Res* 2000; **460**:245-256.
11 Schofield MJ, Hsieh P. DNA mismatch repair: molecular mechanisms and biological function. *Annu Rev Microbiol* 2003; **57**:579-608.
12 Lahue RS, Au KG, Modrich P. DNA mismatch correction in a defined system. *Science* 1989; **245**:160-164.
13 Burdett V, Baitinger C, Viswanathan M, Lovett ST, Modrich P. *In vivo* requirement for RecJ, ExoVII, ExoI, and ExoX in methyl-directed mismatch repair. *Proc Natl Acad Sci USA* 2001; **98**:6765-6770.
14 Obmolova G, Ban C, Hsieh P, Yang W. Crystal structures of mismatch repair protein MutS and its complex with a substrate DNA. *Nature* 2000; **407**:703-710.
15 Lamers MH, Perrakis A, Enzlin JH, Winterwerp HH, de Wind N, Sixma TK. The crystal structure of DNA mismatch repair protein MutS binding to a G•T mismatch. *Nature* 2000; **407**:711-717.
16 Kadyrov FA, Dzantiev L, Constantin N, Modrich P. Endonu-

cleolytic function of MutLalpha in human mismatch repair. *Cell* 2006; **126**:297-308.
17 Sancar A, Hearst JE. Molecular matchmakers. *Science* 1993; **259**:1415-1420.
18 Dao V, Modrich P. Mismatch-, MutS-, MutL-, and helicase II-dependent unwinding from the single-strand break of an incised heteroduplex. *J Biol Chem* 1998; **273**:9202-9207.
19 Guarne A, Ramon-Maiques S, Wolff EM, *et al*. Structure of the MutL C-terminal domain: a model of intact MutL and its roles in mismatch repair. *Embo J* 2004; **23**:4134-4145.
20 Ban C, Yang W. Crystal structure and ATPase activity of MutL: implications for DNA repair and mutagenesis. *Cell* 1998; **95**:541-552.
21 Aronshtam A, Marinus MG. Dominant negative mutator mutations in the mutL gene of Escherichia coli. *Nucleic Acids Res* 1996; **24**:2498-2504.
22 Junop MS, Obmolova G, Rausch K, Hsieh P, Yang W. Composite active site of an ABC ATPase: MutS uses ATP to verify mismatch recognition and authorize DNA repair. *Mol Cell* 2001; **7**:1-12.
23 Li F, Liu Q, Chen YY, *et al*. Escherichia coli mismatch repair protein MutL interacts with the clamp loader subunits of DNA polymerase III. *Mutat Res* 2007 Jul 25; doi:10.1016/j.mrfmmm.2007.07.008.
24 Lopez de Saro FJ, Marinus MG, Modrich P, O'Donnell M. The beta sliding clamp binds to multiple sites within MutL and MutS. *J Biol Chem* 2006; **281**:14340-14349.
25 Ban C, Yang W. Structural basis for MutH activation in E.coli mismatch repair and relationship of MutH to restriction endonucleases. *Embo J* 1998; **17**:1526-1534.
26 Lee JY, Chang J, Joseph N, Ghirlando R, Rao DN, Yang W. MutH complexed with hemi- and unmethylated DNAs: coupling base recognition and DNA cleavage. *Mol Cell* 2005; **20**:155-166.
27 Ramilo C, Gu L, Guo S, *et al*. Partial reconstitution of human DNA mismatch repair *in vitro*: characterization of the role of human replication protein A. *Mol Cell Biol* 2002; **22**:2037-2046.
28 Reenan RA, Kolodner RD. Isolation and characterization of two Saccharomyces cerevisiae genes encoding homologs of the bacterial HexA and MutS mismatch repair proteins. *Genetics* 1992; **132**:963-973.
29 Fishel R, Lescoe MK, Rao MR, *et al*. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. *Cell* 1993; **75**:1027-1038.
30 Leach FS, Nicolaides NC, Papadopoulos N, *et al*. Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. *Cell* 1993; **75**:1215-1225.
31 Palombo F, Gallinari P, Iaccarino I, *et al*. GTBP, a 160-kilodalton protein essential for mismatch-binding activity in human cells. *Science* 1995; **268**:1912-1914.
32 Drummond JT, Li GM, Longley MJ, Modrich P. Isolation of an hMSH2-p160 heterodimer that restores DNA mismatch repair to tumor cells. *Science* 1995; **268**:1909-1912.
33 Bronner CE, Baker SM, Morrison PT, *et al*. Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. *Nature* 1994; **368**:258-261.
34 Li GM, Modrich P. Restoration of mismatch repair to nuclear extracts of H6 colorectal tumor cells by a heterodimer of human MutL homologs. *Proc Natl Acad Sci USA* 1995; **92**:1950-1954.
35 Nicolaides NC, Papadopoulos N, Liu B, *et al*. Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. *Nature* 1994; **371**:75-80.
36 Papadopoulos N, Nicolaides NC, Wei YF, *et al*. Mutation of a mutL homolog in hereditary colon cancer. *Science* 1994; **263**:1625-1629.
37 Schmutte C, Marinescu RC, Sadoff MM, Guerrette S, Overhauser J, Fishel R. Human exonuclease I interacts with the mismatch repair protein hMSH2. *Cancer Res* 1998; **58**:4537-4542.
38 Tishkoff DX, Amin NS, Viars CS, Arden KC, Kolodner RD. Identification of a human gene encoding a homologue of Saccharomyces cerevisiae EXO1, an exonuclease implicated in mismatch repair and recombination. *Cancer Res* 1998; **58**:5027-5031.
39 Tishkoff DX, Boerger AL, Bertrand P, *et al*. Identification and characterization of Saccharomyces cerevisiae EXO1, a gene encoding an exonuclease that interacts with MSH2. *Proc Natl Acad Sci USA* 1997; **94**:7487-7492.
40 Lin YL, Shivji MK, Chen C, Kolodner R, Wood RD, Dutta A. The evolutionarily conserved zinc finger motif in the largest subunit of human replication protein A is required for DNA replication and mismatch repair but not for nucleotide excision repair. *J Biol Chem* 1998; **273**:1453-1461.
41 Gu L, Hong Y, McCulloch S, Watanabe H, Li GM. ATP-dependent interaction of human mismatch repair proteins and dual role of PCNA in mismatch repair. *Nucleic Acids Res* 1998; **26**:1173-1178.
42 Johnson RE, Kovvali GK, Guzder SN, *et al*. Evidence for involvement of yeast proliferating cell nuclear antigen in DNA mismatch repair. *J Biol Chem* 1996; **271**:27987-27990.
43 Umar A, Buermeyer AB, Simon JA, *et al*. Requirement for PCNA in DNA mismatch repair at a step preceding DNA resynthesis. *Cell* 1996; **87**:65-73.
44 Longley MJ, Pierce AJ, Modrich P. DNA polymerase delta is required for human mismatch repair *in vitro*. *J Biol Chem* 1997; **272**:10917-10921.
45 Zhang Y, Yuan F, Presnell SR, *et al*. Reconstitution of 5′-directed human mismatch repair in a purified system. *Cell* 2005; **122**:693-705.
46 Prolla TA, Christie DM, Liskay RM. Dual requirement in yeast DNA mismatch repair for MLH1 and PMS1, two homologs of the bacterial mutL gene. *Mol Cell Biol* 1994; **14**:407-415.
47 Lipkin SM, Wang V, Jacoby R, *et al*. MLH3: a DNA mismatch repair gene associated with mammalian microsatellite instability. *Nat Genet* 2000; **24**:27-35.
48 Bowers J, Tran PT, Joshi A, Liskay RM, Alani E. MSH-MLH complexes formed at a DNA mismatch are disrupted by the PCNA sliding clamp. *J Mol Biol* 2001; **306**:957-968.
49 Clark AB, Valle F, Drotschmann K, Gary RK, Kunkel TA. Functional interaction of proliferating cell nuclear antigen with MSH2-MSH6 and MSH2-MSH3 complexes. *J Biol Chem* 2000; **275**:36498-36501.
50 Flores-Rozas H, Clark D, Kolodner RD. Proliferating cell nuclear antigen and Msh2p-Msh6p interact to form an active mispair recognition complex. *Nat Genet* 2000; **26**:375-378.
51 Kleczkowska HE, Marra G, Lettieri T, Jiricny J. hMSH3 and hMSH6 interact with PCNA and colocalize with it to replication foci. *Genes Dev* 2001; **15**:724-736.

52 Warbrick E. The puzzle of PCNA's many partners. *Bioessays* 2000; **22**:997-1006.
53 Lau PJ, Kolodner RD. Transfer of the MSH2.MSH6 complex from proliferating cell nuclear antigen to mispaired bases in DNA. *J Biol Chem* 2003; **278**:14-17.
54 Shell SS, Putnam CD, Kolodner RD. The N terminus of Saccharomyces cerevisiae Msh6 is an unstructured tether to PCNA. *Mol Cell* 2007; **26**:565-578.
55 Guo S, Presnell SR, Yuan F, Zhang Y, Gu L, Li GM. Differential requirement for proliferating cell nuclear antigen in 5¢ and 3¢ nick-directed excision in human mismatch repair. *J Biol Chem* 2004; **279**:16912-16917.
56 Amin NS, Nguyen MN, Oh S, Kolodner RD. exo1-Dependent mutator mutations: model system for studying functional interactions in mismatch repair. *Mol Cell Biol* 2001; **21**:5142-5155.
57 Nielsen FC, Jager AC, Lutzen A, Bundgaard JR, Rasmussen LJ. Characterization of human exonuclease 1 in complex with mismatch repair proteins, subcellular localization and association with PCNA. *Oncogene* 2004; **23**:1457-1468.
58 Tran PT, Erdeniz N, Symington LS, Liskay RM. EXO1-A multi-tasking eukaryotic nuclease. *DNA Repair (Amst)* 2004; **3**:1549-1559.
59 Genschel J, Modrich P. Mechanism of 5′-directed excision in human mismatch repair. *Mol Cell* 2003; **12**:1077-1086.
60 Dzantiev L, Constantin N, Genschel J, Iyer RR, Burgers PM, Modrich P. A defined human system that supports bidirectional mismatch-provoked excision. *Mol Cell* 2004; **15**:31-41.
61 Genschel J, Bazemore LR, Modrich P. Human exonuclease I is required for 5′ and 3′ mismatch repair. *J Biol Chem* 2002; **277**:13302-13311.
62 Wei K, Clark AB, Wong E, *et al*. Inactivation of Exonuclease 1 in mice results in DNA mismatch repair defects, increased cancer susceptibility, and male and female sterility. *Genes Dev* 2003; **17**:603-614.
63 Guo S, Zhang Y, Yuan F, *et al*. Regulation of replication protein A functions in mismatch repair by phosphorylation. *J Biol Chem*, 2006; **281**:21607-21616.
64 Fleck O, Kunz C, Rudolph C, Kohli J. The high mobility group domain protein Cmb1 of Schizosaccharomyces pombe binds to cytosines in base mismatches and opposite chemically altered guanines. *J Biol Chem* 1998; **273**:30398-30405.
65 Javaherian K, Liu JF, Wang JC. Nonhistone proteins HMG1 and HMG2 change the DNA helical structure. *Science* 1978; **199**:1345-1346.
66 Javaherian K, Sadeghi M, Liu LF. Nonhistone proteins HMG1 and HMG2 unwind DNA double helix. *Nucleic Acids Res* 1979; **6**:3569-3580.
67 Yuan F, Gu L, Guo S, Wang C, Li GM. Evidence for involvement of HMGB1 protein in human DNA mismatch repair. *J Biol Chem* 2004; **279**:20935-20940.
68 Wang H, Hays JB. Signaling from DNA mispairs to mismatch-repair excision sites despite intervening blockades. *Embo J* 2004; **23**:2126-2133.
69 Allen DJ, Makhov A, Grilley M, *et al*. MutS mediates heteroduplex loop formation by a translocation mechanism. *Embo J* 1997; **16**:4467-4476.
70 Fishel R. Mismatch repair, molecular switches, and signal transduction. *Genes Dev* 1998; **12**:2096-2101.
71 Gradia S, Acharya S, Fishel R. The human mismatch recognition complex hMSH2-hMSH6 functions as a novel molecular switch. *Cell* 1997; **91**:995-1005.
72 Jiang J, Bai L, Surtees JA, Gemici Z, Wang MD, Alani E. Detection of high-affinity and sliding clamp modes for MSH2-MSH6 by single-molecule unzipping force analysis. *Mol Cell* 2005; **20**:771-781.
73 Mendillo ML, Mazur DJ, Kolodner RD. Analysis of the interaction between the Saccharomyces cerevisiae MSH2-MSH6 and MLH1-PMS1 complexes with DNA using a reversible DNA end-blocking system. *J Biol Chem* 2005; **280**:22245-22257.
74 Pluciennik A, Modrich P. From the cover: protein roadblocks and helix discontinuities are barriers to the initiation of mismatch repair. *Proc Natl Acad Sci USA* 2007; **104**:12709-12713.
75 Au KG, Welsh K, Modrich P. Initiation of methyl-directed mismatch repair. *J Biol Chem* 1992; **267**:12142-12148.
76 Kat A, Thilly WG, Fang WH, Longley MJ, Li GM, Modrich P. An alkylation-tolerant, mutator human cell line is deficient in strand-specific mismatch repair. *Proc Natl Acad Sci USA* 1993; **90**:6424-6428.
77 Koi M, Umar A, Chauhan DP, *et al*. Human chromosome 3 corrects mismatch repair deficiency and microsatellite instability and reduces *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine tolerance in colon tumor cells with homozygous hMLH1 mutation. *Cancer Res* 1994; **54**:4308-4312.
78 Fink D, Nebel S, Norris PS, *et al*. The effect of different chemotherapeutic agents on the enrichment of DNA mismatch repair-deficient tumour cells. *Br J Cancer* 1998; **77**:703-708.
79 Fujii H, Shimada T. Isolation and characterization of cDNA clones derived from the divergently transcribed gene in the region upstream from the human dihydrofolate reductase gene. *J Biol Chem* 1989; **264**:10057-10064.
80 Linton JP, Yen J-YJ, Selby E, *et al*. Dual bidirectional promoters at the mouse *DHFR* locus: cloning and characterization of two mRNA classes of the divergently transcribed *Rep-1* gene. *Mol Cell Biol* 1989; **9**:3058-3072.
81 Drummond JT, Genschel J, Wolf E, Modrich P. DHFR/MSH3 amplification in methotrexate-resistant cells alters the hMutSalpha/hMutSbeta ratio and reduces the efficiency of base-base mismatch repair. *Proc Natl Acad Sci USA* 1997; **94**:10144-10149.
82 Marra G, Iaccarino I, Lettieri T, Roscilli G, Delmastro P, Jiricny J. Mismatch repair deficiency associated with overexpression of the MSH3 gene. *Proc Natl Acad Sci USA* 1998; **95**:8568-8573.
83 Brown KD, Rathi A, Kamath R, *et al*. The mismatch repair system is required for S-phase checkpoint activation. *Nat Genet* 2003; **33**:80-84.
84 Duckett DR, Drummond JT, Murchie AI, *et al*. Human MutSalpha recognizes damaged DNA base pairs containing O6-methylguanine, O4-methylthymine, or the cisplatin-d(GpG) adduct. *Proc Natl Acad Sci USA* 1996; **93**:6443-6447.
85 Gong JG, Costanzo A, Yang HQ, *et al*. The tyrosine kinase c-Abl regulates p73 in apoptotic response to cisplatin-induced DNA damage. *Nature* 1999; **399**:806-809.
86 Stojic L, Mojas N, Cejka P, *et al*. Mismatch repair-dependent G2 checkpoint induced by low doses of SN1 type methylating agents requires the ATR kinase. *Genes Dev* 2004; **18**:1331-1344.
87 Kim WJ, Rajasekaran B, Brown KD. MLH1 and ATM-dependent MAP kinase signaling is activated through C-Abl in response to the alkylator N-methyl-N′-nitro-N-nitrosoguanidine.

J Biol Chem, 2007; **282**:32021-32031.
88 Shimodaira H, Yoshioka-Yamashita A, Kolodner RD, Wang JY. Interaction of mismatch repair protein PMS2 and the p53-related transcription factor p73 in apoptosis response to cisplatin. *Proc Natl Acad Sci USA* 2003; **100**:2420-2425.
89 Yoshioka K, Yoshioka Y, Hsieh P. ATR kinase activation mediated by MutSalpha and MutLalpha in response to cytotoxic O6-methylguanine adducts. *Mol Cell* 2006; **22**:501-510.
90 Schaetzlein S, Kodandaramireddy NR, Ju Z, *et al*. Exonuclease-1 deletion impairs DNA damage signaling and prolongs lifespan of telomere-dysfunctional mice. *Cell* 2007; **130**:863-877.
91 York SJ, Modrich P. Mismatch repair-dependent iterative excision at irreparable O6-methylguanine lesions in human nuclear extracts. *J Biol Chem* 2006; **281**:22674-22683.
92 Kolodner RD, Putnam CD, Myung K. Maintenance of genome stability in Saccharomyces cerevisiae. *Science* 2002; **297**:552-557.
93 Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. *Nat Med* 2004; **10**:789-799.
94 Harfe BD, Jinks-Robertson S. DNA mismatch repair and genetic instability. *Annu Rev Genet* 2000; **34**:359-399.
95 Worth L Jr, Clark S, Radman M, Modrich P. Mismatch repair proteins MutS and MutL inhibit RecA-catalyzed strand transfer between diverged DNAs. *Proc Natl Acad Sci USA* 1994; **91**:3238-3241.
96 Goldfarb T, Alani E. Distinct roles for the Saccharomyces cerevisiae mismatch repair proteins in heteroduplex rejection, mismatch repair and nonhomologous tail removal. *Genetics* 2005; **169**:563-574.
97 Sugawara N, Goldfarb T, Studamire B, Alani E, Haber JE. Heteroduplex rejection during single-strand annealing requires Sgs1 helicase and mismatch repair proteins Msh2 and Msh6 but not Pms1. *Proc Natl Acad Sci USA* 2004; **101**:9315-9320.
98 Myung K, Datta A, Chen C, Kolodner RD. SGS1, the Saccharomyces cerevisiae homologue of BLM and WRN, suppresses genome instability and homeologous recombination. *Nat Genet* 2001; **27**:113-116.
99 Doherty KM, Sharma S, Uzdilla L, *et al*. RECQ1 helicase interacts with human mismatch repair factors that regulate genetic recombination. *J Biol Chem*, 2005; **280**:28085-28094.
100 Pedrazzi G, Bachrati CZ, Selak N, *et al*. The Bloom¢s syndrome helicase interacts directly with the human DNA mismatch repair protein hMSH6. *Biol Chem* 2003; **384**:1155-1164.
101 Peng M, Litman R, Xie J, Sharma S, Brosh RM Jr, Cantor SB. The FANCJ/MutLalpha interaction is required for correction of the cross-link response in FA-J cells. *Embo J* 2007; **26**:3238-3249.
102 Zhang N, Liu X, Li L, Legerski R. Double-strand breaks induce homologous recombinational repair of interstrand cross-links via cooperation of MSH2, ERCC1-XPF, REV3, and the Fanconi anemia pathway. *DNA Repair (Amst)* 2007; **6**:1670-1678.
103 Zhang N, Lu X, Zhang X, Peterson CA, Legerski RJ. hMutSbeta is required for the recognition and uncoupling of psoralen interstrand cross-links *in vitro*. *Mol Cell Biol* 2002; **22**:2388-2397.
104 Gu L, Wu J, Qiu L, Jennings CD, Li GM. Involvement of DNA mismatch repair in folate deficiency-induced apoptosis small star, filled. *J Nutr Biochem* 2002; **13**:355-363.
105 Wilson TM, Vaisman A, Martomo SA, *et al*. MSH2-MSH6 stimulates DNA polymerase eta, suggesting a role for A:T mutations in antibody genes. *J Exp Med* 2005; **201**:637-645.
106 Casali P, Pal Z, Xu Z, Zan H. DNA repair in antibody somatic hypermutation. *Trends Immunol* 2006; **27**:313-321.
107 Schrader CE, Guikema JE, Linehan EK, Selsing E, Stavnezer J. Activation-induced cytidine deaminase-dependent DNA breaks in class switch recombination occur during G1 phase of the cell cycle and depend upon mismatch repair. *J Immunol* 2007; **179**:6064-6071.
108 Bardwell PD, Woo CJ, Wei K, *et al*. Altered somatic hypermutation and reduced class-switch recombination in exonuclease 1-mutant mice. *Nat Immunol* 2004; **5**:224-229.
109 Winter DB, Phung QH, Umar A, *et al*. Altered spectra of hypermutation in antibodies from mice deficient for the DNA mismatch repair protein PMS2. *Proc Natl Acad Sci USA* 1998; **95**:6953-6958.
110 Pearson CE, Nichol Edamura K, Cleary JD. Repeat instability: mechanisms of dynamic mutations. *Nat Rev Genet* 2005; **6**:729-742.
111 Gacy AM, Goellner G, Juranic N, Macura S, McMurray CT. Trinucleotide repeats that expand in human disease form hairpin structures *in vitro*. *Cell* 1995; **81**:533-540.
112 Manley K, Shirley TL, Flaherty L, Messer A. Msh2 deficiency prevents *in vivo* somatic instability of the CAG repeat in Huntington disease transgenic mice. *Nat Genet* 1999; **23**:471-473.
113 Owen BA, Yang Z, Lai M, *et al*. (CAG)(n)-hairpin DNA binds to Msh2-Msh3 and changes properties of mismatch recognition. *Nat Struct Mol Biol* 2005; **12**:663-670.
114 Aaltonen LA, Peltomaki P, Leach FS, *et al*. Clues to the pathogenesis of familial colorectal cancer. *Science* 1993; **260**:812-816.
115 Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M. Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. *Nature* 1993; **363**:558-561.
116 Thibodeau SN, Bren G, Schaid D. Microsatellite instability in cancer of the proximal colon. *Science* 1993; **260**:816-819.
117 Strand M, Prolla TA, Liskay RM, Petes TD. Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. *Nature* 1993; **365**:274-276.
118 Parsons R, Li GM, Longley MJ, *et al*. Hypermutability and mismatch repair deficiency in RER+ tumor cells. *Cell* 1993; **75**:1227-1236.
119 Umar A, Boyer JC, Thomas DC, *et al*. Defective mismatch repair in extracts of colorectal and endometrial cancer cell lines exhibiting microsatellite instability. *J Biol Chem* 1994; **269**:14367-14370.
120 Lindblom A, Tannergard P, Werelius B, Nordenskjold M. Genetic mapping of a second locus predisposing to hereditary non-polyposis colon cancer. *Nat Genet* 1993; **5**:279-282.
121 Li GM. DNA mismatch repair and cancer. *Front Biosci* 2003; **8**:d997-d1017.
122 Liberti SE, Rasmussen LJ. Is hEXO1 a cancer predisposing gene? *Mol Cancer Res* 2004; **2**:427-432.
123 Sun X, Zheng L, Shen B. Functional alterations of human exonuclease 1 mutants identified in atypical hereditary nonpolyposis colorectal cancer syndrome. *Cancer Res* 2002; **62**:6026-6030.
124 Boland CR, Thibodeau SN, Hamilton SR, *et al*. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of interna-

tional criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 1998; **58**:5248-5257.
125 Gu L, Cline-Brown B, Zhang F, Qiu L, Li GM. Mismatch repair deficiency in hematological malignancies with microsatellite instability. *Oncogene* 2002; **21**:5758-5764.
126 Gu L, Wu J, Zhu BB, Li GM. Deficiency of a novel mismatch repair activity in a bladder tumor cell line. *Nucleic Acids Res* 2002; **30**:2758-2763.
127 Prolla TA, Baker SM, Harris AC, *et al*. Tumour susceptibility and spontaneous mutation in mice deficient in Mlh1, Pms1 and Pms2 DNA mismatch repair. *Nat Genet* 1998; **18**:276-279.
128 Wei K, Kucherlapati R, Edelmann W. Mouse models for human DNA mismatch-repair gene defects. *Trends Mol Med* 2002; **8**:346-353.
129 de Wind N, Dekker M, Berns A, Radman M, te Riele H. Inactivation of the mouse Msh2 gene results in mismatch repair deficiency, methylation tolerance, hyperrecombination, and predisposition to cancer. *Cell* 1995; **82**:321-330.
130 Reitmair AH, Schmits R, Ewel A, *et al*. MSH2 deficient mice are viable and susceptible to lymphoid tumours. *Nat Genet* 1995; **11**:64-70.
131 Edelmann W, Yang K, Umar A, *et al*. Mutation in the mismatch repair gene Msh6 causes cancer susceptibility. *Cell* 1997; **91**:467-477.
132 Kolodner RD, Tytell JD, Schmeits JL, *et al*. Germ-line msh6 mutations in colorectal cancer families. *Cancer Res* 1999; **59**:5068-5074.
133 de Wind N, Dekker M, Claij N, *et al*. HNPCC-like cancer predisposition in mice through simultaneous loss of Msh3 and Msh6 mismatch-repair protein functions. *Nat Genet* 1999; **23**:359-362.
134 Edelmann W, Umar A, Yang K, *et al*. The DNA mismatch repair genes Msh3 and Msh6 cooperate in intestinal tumor suppression. *Cancer Res* 2000; **60**:803-807.
135 Baker SM, Plug AW, Prolla TA, *et al*. Involvement of mouse Mlh1 in DNA mismatch repair and meiotic crossing over. *Nat Genet* 1996; **13**:336-342.
136 Edelmann W, Cohen PE, Kane M, *et al*. Meiotic pachytene arrest in MLH1-deficient mice. *Cell* 1996; **85**:1125-1134.
137 Lipkin SM, Moens PB, Wang V, *et al*. Meiotic arrest and aneuploidy in MLH3-deficient mice. *Nat Genet* 2002; **31**:385-390.
138 Baker SM, Bronner CE, Zhang L, *et al*. Male mice defective in the DNA mismatch repair gene PMS2 exhibit abnormal chromosome synapsis in meiosis. *Cell* 1995; **82**:309-319.
139 Kane MF, Loda M, Gaida GM, *et al*. Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. *Cancer Res* 1997; **57**:808-811.
140 Grady WM, Markowitz SD. Genetic and epigenetic alterations in colon cancer. *Annu Rev Genomics Hum Genet* 2002; **3**:101-128.
141 Thibodeau SN, French AJ, Cunningham JM, *et al*. Microsatellite instability in colorectal cancer: different mutator phenotypes and the principal involvement of hMLH1. *Cancer Res* 1998; **58**:1713-1718.
142 Gazzoli I, Loda M, Garber J, Syngal S, Kolodner RD. A hereditary nonpolyposis colorectal carcinoma case associated with hypermethylation of the MLH1 gene in normal tissue and loss of heterozygosity of the unmethylated allele in the resulting microsatellite instability-high tumor. *Cancer Res* 2002; **62**:3925-3928.
143 Chan TL, Yuen ST, Kong CK, *et al*. Heritable germline epimutation of MSH2 in a family with hereditary nonpolyposis colorectal cancer. *Nat Genet* 2006; **38**:1178-1183.
144 Hitchins MP, Wong JJ, Suthers G, *et al*. Inheritance of a cancer-associated MLH1 germ-line epimutation. *N Engl J Med* 2007; **356**:697-705.
145 Suter CM, Martin DI, Ward RL. Germline epimutation of MLH1 in individuals with multiple cancers. *Nat Genet* 2004; **36**:497-501.
146 Veigl ML, Kasturi L, Olechnowicz J, *et al*. Biallelic inactivation of hMLH1 by epigenetic gene silencing, a novel mechanism causing human MSI cancers. *Proc Natl Acad Sci USA* 1998; **95**:8698-8702.
147 Herman JG, Umar A, Polyak K, *et al*. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. *Proc Natl Acad Sci USA* 1998; **95**:6870-6875.
148 Kinzler KW, Vogelstein B. Cancer-susceptibility genes. Gatekeepers and caretakers. *Nature* 1997; **386**:761, 763.
149 Markowitz S, Wang J, Myeroff L, *et al*. Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. *Science* 1995; **268**:1336-1338.
150 Duval A, Hamelin R. Mutations at coding repeat sequences in mismatch repair-deficient human cancers: toward a new concept of target genes for instability. *Cancer Res* 2002; **62**:2447-2454.