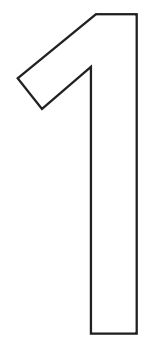
General Introduction: The Genetics and Biochemistry of DNA Mismatch Repair and Implications for Carcinogenesis



Genome Stability

DNA repair pathways protect cells from genomic instability, and the organism from aging and cancer. To preserve the information encoded by the DNA of a cell it is essential to prevent mutations. Mutations can arise as a consequence of unrepaired exogenous and endogenous damage to DNA. A plethora of mechanisms is available to cells to either repair or tolerate DNA damages (reviewed in [Hoeijmakers, 2009; Jackson and Bartek, 2009; Ciccia and Elledge, 2010]. Though DNA damage is a major threat to the genome, its integrity can also be threatened by DNA replication. The fidelity of DNA replication is ensured by several factors. First, the main replicative polymerases δ and ε display a high degree of nucleotide selectivity, misincorporating at a frequency of only approximately 10^{-4} to 10^{-5} [Iyer et al., 2006; Arana and Kunkel, 2010]. Then, the proofreading activities of polymerases δ and ε can remove most misincorporations, increasing replication fidelity to 10^{-7} per basepair per replication round. Residual misincorporations are detected and corrected by the DNA mismatch repair (MMR) system. The orchestrated action of these pathways ensures extremely accurate replication fidelity [Arana and Kunkel, 2010], and protects organisms from cancer development [Hanahan and Weinberg, 2000, 2011].

DNA Mismatch Repair

MMR is an evolutionary conserved postreplicative DNA repair system that corrects (spontaneous) replication errors, such as base-base mispairs and insertion/deletion loops (IDLs; reviewed in [Hsieh and Yamane, 2008; Li, 2008; Jiricny, 2013]). Besides its role in replication fidelity, MMR has also been implicated in homologous recombination, in somatic hyper mutation, in triplet repeat expansion, crosslink repair and in the response to various DNA damaging agents such as methylating agents, oxidative agents, ionizing radiation and nucleotide analogs [Stojic et al., 2004; McMurray, 2010; Deans and West, 2011; Jiricny, 2013 and references therein].

During the repair of replication errors in eukaryotes, the MMR pathway is initiated by the recognition of a mismatch or IDL by either the heterodimeric MutSα, consisting of the homologous MSH2 and MSH6 proteins, or the MutSβ heterodimer consisting of MSH2 and MSH3. These early factors in MMR are named after their bacterial homolog MutS (MSH=MutS Homolog) that was first described over 40 years ago [Cox et al., 1972]. The MutSα complex is responsible for the recognition of base-base mispairs and small IDLs, whereas MutSβ is responsible for the redundant repair mainly of larger IDLs. Each subunit of MutSα and MutSβ consists of five major domains, which have been referred to as a mismatch binding domain, a connector domain, a lever domain, a clamp domain and an ATPase domain (Figure 1A and B; [Warren et al., 2007; Gupta et al., 2012]). These domain names are analogous to the domains described in the bacterial MutS [Lamers et al., 2000; Obmolova et al., 2000]. The term ”mismatch binding domain” is, in fact, a misnomer for MSH2 and MSH3, as only the mismatch binding in MSH6 actually binds mismatches. Both the MutSα and the MutSβ complex very much resembe a Θ-like structure. The (mis) matched DNA is in the upper channel, the lower channel is empty, and MSH2 and MSH6 line the sides along the long axis (Figure 1C; [Warren et al., 2007; Gupta et al., 2012]).

The MutSα heterodimer is recruited onto chromatin through a PWWP motif in the N-terminus of MSH6, by interacting with the histone mark H3K56me3 [Li et al., 2013]. In addition, MutSα complexes are targeted to newly synthesized DNA by being a constitutive part of replication factories in a manner depending on an interaction with PCNA (Figure 2; [Iyer et al., 2008; Hombauer et al., 2011a]). Also, MMR proteins only function when expressed during replication and not after, in support of the coupling between replication and MMR [Hombauer et al., 2011b]. Upon mismatch binding, MSH6 makes contact with the mismatch through a conserved Phe-X-Glu motif in the mismatch binding domain in its N-terminus. The phenylalanine residue is inserted into the DNA helix and stacks against the mispaired nucleotide, which results in kinking of the DNA by 60° [Lamers et al., 2000; Obmolova et al., 2000; Warren et al., 2007]. MSH3 lacks a Phe-X-Glu motif but it contacts the backbone of the DNA through several amino acids its N-terminus [Gupta et al., 2012].

All MutS homologs are ATPases that carry a Walker ATP motif, a common motif among DNA repair proteins, in their C-terminal ATPase domains. They are members of the ATP Binding Cassette (ABC) transporter ATPase superfamily (Figure 1A; reviewed in [Holland and Blight, 1999]). Each MutS homolog heterodimer has two composite nucleotide binding sites, one on each subunit. Both contain a Walker A and Walker B motif from one subunit, and an ABC signature motif from the other subunit. The binding sites are nonidentical: in MutSα, MSH6 has high affinity for ATP, whereas MSH2 has a higher affinity for ADP [Antony and Hingorani, 2003; Antony et al., 2006]. After the MutSα complex binds a mismatch through its mismatch binding domain, the complex exchanges ADP for ATP and undergoes an ATP-induced conformational change, converting the heterodimer into a sliding clamp [Gradia et al., 1999], which can freely move along the DNA helix contour [Gorman et al., 2007].

After DNA mismatch binding MutSα (or MutSβ) recruits the MutLα heterodimer (consisting of MLH1-PMS2; Figure 2). This complex is thought to act as a matchmaker protein between the MutSα mismatch recognizing complex and the downstream exonuclease. Both MLH1 and PMS2 contain an N-terminal ATPase domain and a C-terminal dimerization domain, connected by a linker (Figure 1C). MLH1 (Mut L Homolog 1) belongs to the superfamily of GKHL (gyrase II/Hsp90/histidine kinase/MutL) ATPases (Figure 1C and D). MutLα is partially redundant with another heterodimer, MutLγ (consisting of MLH1-MLH3). However, MutLγ cannot fully substitute for MutLα, at least *in vitro* [Cannavo et al., 2005; Korhonen et al., 2008]. The MutLα complex helps to stabilize the ternary DNA·MutSα·MutLα complex. Like MutSα, MutLα has (weak) ATPase activity and undergoes sequential conformational changes after ATP binding (Figure 1C; [Sacho et al., 2008]), but the relevance of these changes is still very poorly understood.

Besides functioning as a matchmaker, MutLα is very important because of the endonuclease activity within the C-terminus of the PMS2 (Postmeiotic segregation increased 2) subunit. To direct MMR-mediated excision to the newly synthesized strand, MMR requires a strand discrimination signal that *in vitro* is a nick [Lahue et al., 1989; Constantin et al., 2005]. It has been proposed that lagging strand MMR is directed, at least in part, by 3' or 5' ends of Okazaki fragments [Pavlov et al., 2003]. MMR at the leading strand, in turn, partly depends

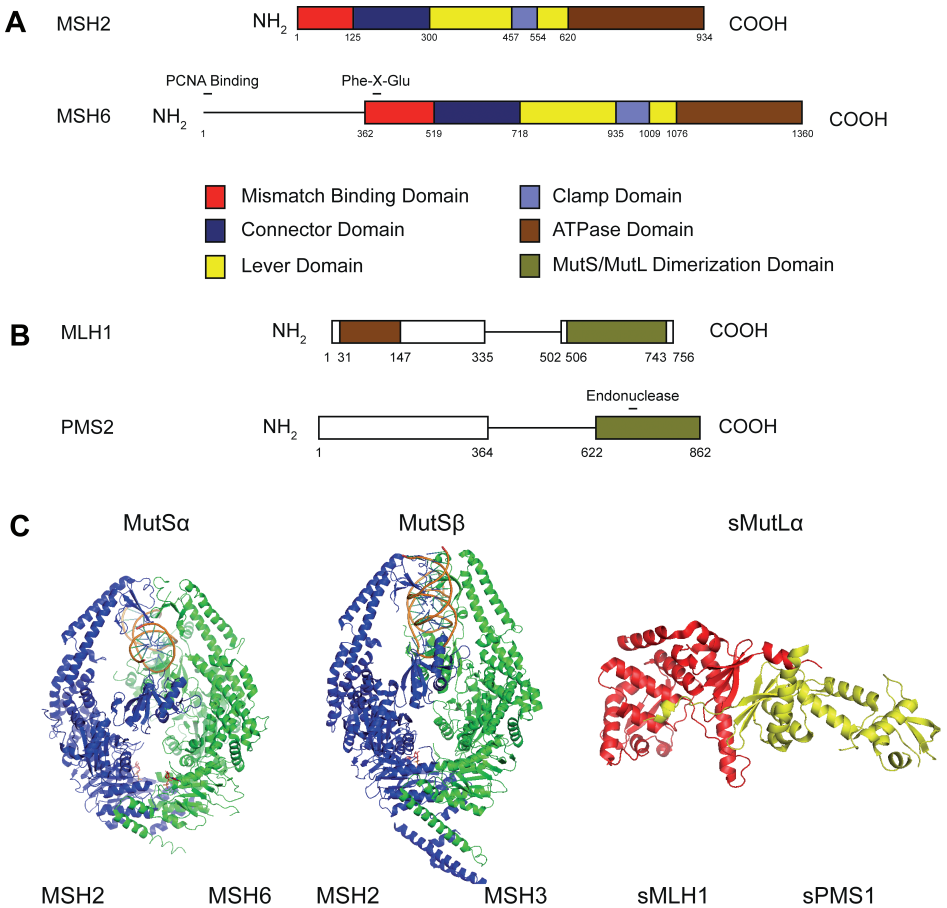


Figure 1. *Domains and crystal structures of mismatch repair proteins and complexes. (A) Schematic representation of domains in the primary protein structures of MSH2 and MSH6. Numbering reflects amino acid numbering. Domains are as published [Warren et al., 2007]. Of note, the mismatch binding domain in MSH2 does not bind mismatches (see main text). PCNA binding: PCNA binding motif [Hombauer et al., 2011a]. Phe-X-Glu: Mismatch binding motif. (B) As A, but for MLH1 and PMS2. Domains have been described in [Raevaara et al., 2005; Gueneau et al., 2013]. Endonuclease: Endonuclease motif [Kadyrov et al., 2006]. (C) Crystal structures of the human MutSα (MSH2/MSH6; [Warren et al., 2007]), human MutSβ (MSH2/MSH3; [Gupta et al., 2012]) and S. cerevisiae MutLα (MLH1/PMS1, only the C-terminal domain is shown as only this part has been crystallized, now [Gueneau et al., 2013]).*

on small gaps induced after the removal of ribonucleotides from the nascent strand by RNase H2, which are fortuitously incorporated during replication by the leading strand polymerase ε [Ghodgaonkar et al., 2013; Lujan et al., 2013]. In addition to these RNase H2-induced gaps, additional signals may serve as strand a discrimination, such as the 3' terminus of a leading strand. The 3' terminus at the nascent strand dictates the polarity of the processivity factor Proliferating Cell Nuclear Antigen (PCNA) on DNA, which in turn determines the directionality of the nicking activity of MutLα on DNA [Peña-Diaz and Jiricny, 2010; Pluciennik

et al., 2010]. The endonuclease activity of the PMS2 subunit introduces new nicks on the nascent strand, 5' of the mismatch [Kadyrov et al., 2006]. Such nicks serve as entry point for Exonuclease 1 (EXO1), a 5'-3' exonuclease that carries out excision of the misincorporation-containing DNA strand. *In vitro* reconstitution of MMR depends on EXO1 [Dzantiev et al., 2004; Constantin et al., 2005] but, oddly enough, *Exo1*$^{-/-}$ cells have a less severe phenotype than *Msh2*$^{-/-}$ cells [Wei et al., 2003], suggesting that other exonucleases or mechanisms may substitute for EXO1 function. It has recently been shown that MMR, at least *in vitro*, can also occur independently from EXO1 through strand displacement by Polδ (Figure 2; [Kadyrov et al., 2009]). In addition, EXO1 is preferentially required for lagging strand MMR and not for leading strand MMR [Hombauer et al., 2011a], suggesting mechanistically distinct MMR events that differentially require EXO1. As the lagging strand provides an ideal substrate both for strand displacement and EXO1-mediated degradation, this latter finding is surprising.

Once the misincorporation-containing DNA strand is excised, the single stranded DNA patch is protected by the single-strand DNA binding protein Replication Protein A (RPA) and the strand is resynthesized by Polδ in the presence the clamp loader Replication Factor

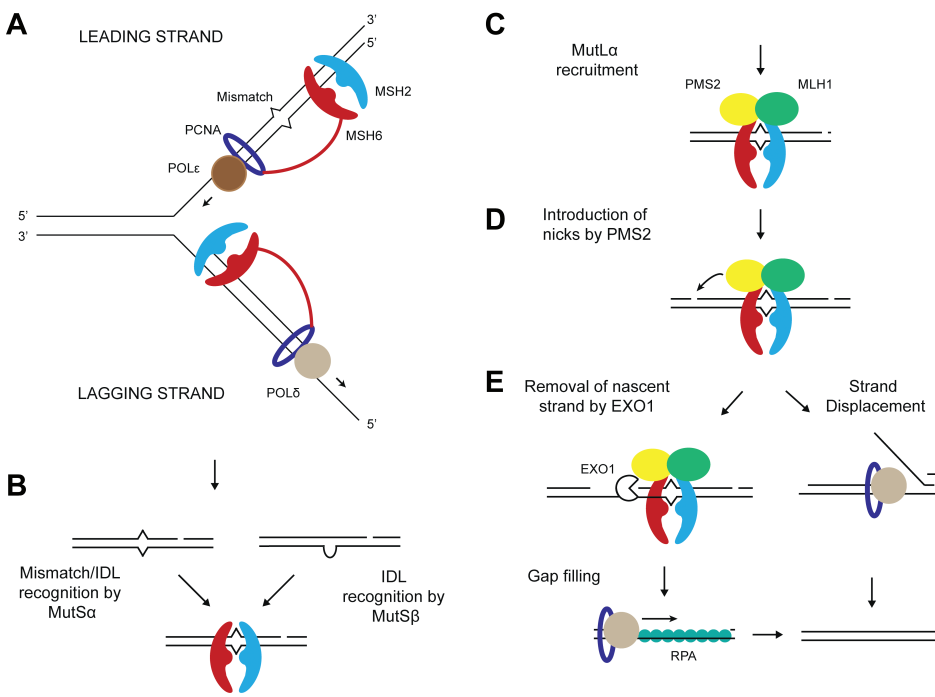


Figure 2. *Sequential steps of canonical MMR. (A) MutSα is a constitutive part of replication factories through an interaction between the N-terminus of MSH6 and PCNA. (B) Mismatches are recognized by MutSα (left panel), while IDLs are recognized mostly by MutSβ. (C) Once a substrate is bound, the MutLα heterodimer is recruited. (D) The PMS2 subunit introduces nicks 5' to the mismatch, creating a substrate for either strand displacement by POLδ or for EXO1-mediated strand removal. After the former, the flap is removed by a flap endonuclease. (E) After the latter, the resulting gap is filled by RFC, PCNA and POLδ, the nick is ligated, and repair is completed.*

C (RFC) that loads PCNA (Figure 2; [Longley et al., 1997; Dzantiev et al., 2004; Constantin et al., 2005]). Additional proteins possibly involved in MMR are HMGB1 [Yuan et al., 2004; Genschel and Modrich, 2009], RFX [Zhang et al., 2008] and PARP1 [Liu et al., 2011], but their roles are more elusive.

Discrimination by MutSα between matched and mismatched DNA *in vitro* is only ~5-30 fold [Hays et al., 2005] and of all mismatches, only the G·T mismatch is bound well by MutSα in Electrophoretic Mobility Shift Assays [Jiricny et al., 1988]. However, in *in vitro* MMR assays, all mismatches are repaired when embedded in perfectly paired DNA [Hays et al., 2005]. Repair of the C·C mismatch is less efficient, but this mismatch is very efficiently removed by the proofreading activity of the replicative polymerases. Overall, the mechanistic basis of detecting mismatches in a large excess of matched DNA is still poorly understood. Mismatch discrimination may be a very complex process, ensured by several (kinetic) mechanisms and/or additional MMR factors [Labazi et al., 2009]. Multiple (ATP-controlled) conformational changes in MutSα may contribute to specificity. Also, the ATP-induced sliding along the DNA may provide only a short time window for MutLα to bind, providing a "kinetic control" of heteroduplex binding. Furthermore, ATP-induced conformational changes in MutLα may also contribute to mismatch specificity [Sacho et al., 2008].

Mutator Phenotype and Microsatellite Instability

MMR increases replication fidelity by 100 to 1000 fold to one replication error per 10^9-10^{10} nucleotides, and in this way protects cells from spontaneous mutations [Iyer et al., 2006]. Vice versa, the inactivation of MMR leads to a mutator phenotype in which spontaneous mutation rates are highly elevated. These elevated mutation rates affect the whole genome, including protein-encoding genes.

MMR deficiency also results in microsatellite instability (MSI). MSI refers to size changes, often shrinkage, of genomic repetitive sequences (such as $[CA]_N$, or $[A]_N$; reviewed in [Boland and Goel, 2010]). This hyper mutable phenotype is caused by low processivity of the replicative polymerases on microsatellites. During replication of these loci, polymerases often have to be reloaded, a process that frequently induces IDLs. These IDLs are a substrate for MMR, which aids in the maintenance of the stability of microsatellites [Strand et al., 1993].

Tolerance of Genotoxic Agents

Besides recognizing mismatches and IDLs, MutSα is also capable of detecting and binding modified DNA bases, such as those induced by oxygen radicals [Mazurek et al., 2002; Macpherson et al., 2005], methylating agents [Duckett et al., 1996; Rasmussen and Samson, 1996] or UV (but only when mispaired [Hoffman et al., 2005]). Cells deficient for MMR are tolerant to various genotoxic agents, such as the methylating agents *N*-Methyl-*N*-nitroso-*N'*-nitroguanidine (MNNG) and Temozolomide, but also to nucleotide analogs such as 6-thioguanine (6-TG; [Swann et al., 1996]) and Fluorouracil (5-FU; [Meyers et al., 2003]).

Of these drugs, the response of MMR proficient and deficient cells to the methylating agent MNNG has received much attention. This agent gives rise to O^6-methylguanines (amongst

others), a highly mutagenic lesion due to its efficient basepairing with thymine [Warren et al., 2006]. The finding that O^6-methylguanines are the toxic lesions is substantiated by the fact that the detoxifying protein methylguanine-DNA methyltransferase (MGMT) protects the cells against MNNG-induced apoptosis [Kaina et al., 2007]. MMR deficient cells are up to a 100 fold more tolerant to MNNG compared with MMR proficient cells [Goldmacher et al., 1986; Kat et al., 1993; Stojic et al., 2005]. The investigation of the MMR-mediated response to MNNG has yielded two major models, that are not mutually exclusive, to explain these phenotypes. In the first model, generally referred to as the direct signaling model, the MMR-mediated response to methylating agents may be regulated in a direct manner, in the absence of MMR-mediated excision. *In vitro* the signaling kinase ATR/ATRIP, but not RPA, is preferentially recruited to O^6meG·T mismatches on a covalently closed circular substrate in a MutSα and MutLα dependent manner. Also, ATR is activated to phosphorylate its target CHK1 in the presence of O^6-meG·T mismatches and MMR proteins *in vitro* in the absence of excision, suggesting direct activation of signaling proteins (Figure 3, right panel; [Yoshioka et al., 2006]). This may be further supported by cellular data showing interactions between MutSα and ATR [Liu et al., 2009; Pabla et al., 2011], TopBP1, Claspin and CHK1, and between MutLα and TopBP1 and Claspin [Liu et al., 2009]. Liu et. al also showed binding of ATR, TopBP1 and CHK1, but not RPA, to chromatin after MNNG treatment in a MutSα and MutLα dependent manner. Oddly enough, this paper only shows recruitment of these factors but not their activation nor contribution in cell cycle responses. An interesting experiment would be to find/generate mutant MMR proteins that do not bind the signaling proteins, and see whether these interactions are required to induce apoptosis in response to MNNG.

In case the direct signaling model is true, one would predict that DNA damage signaling and apoptosis are induced in the first cell cycle after treatment. In contrast to this, DNA damage signaling, G2 arrest and apoptosis are induced in the second cell cycle after treatment [Stojic et al., 2004; Mojas et al., 2007]. This finding is the basis of a second model, referred to as the futile cycling model. Since the O^6-meG cannot properly form a Watson-Crick basepair with any other nucleotide, basepairing of O^6-meG will often yield an MMR substrate. However, since MMR is directed only towards the newly synthesized strand, not the O^6-meG in the parental strand, but its mispairing partner in the nascent strand is removed. Polymerase-mediated gap filling of this structure yields yet another MMR substrate, inducing multiple rounds of MMR [York and Modrich, 2006]. The notion that actual excision of the mismatch is required is supported by the fact that O^6-meG induced cell death requires (the catalytic function of) EXO1 [Klapacz et al., 2009; Schaetzlein et al., 2013]. After several attempts of futile repair, iterative MMR-induced gaps opposite the O^6-meG are believed to be carried over to a subsequent S-phase, in which they becomes toxic DNA intermediates that will induce double-strand breaks when replicated (Figure 3, left panel; [Stojic et al., 2005; Mojas et al., 2007]). The idea that double strand breaks are the inducer of cell death in response to methylating agents is supported by the finding that both ATM [Debiak et al., 2004] and BRCA1 [Yamane et al., 2007] are involved in the cellular response to methylation damage and that *S. cerevisiae* is relatively tolerant to MNNG-induced cell death due to efficient recombination [Cejka et al., 2005].

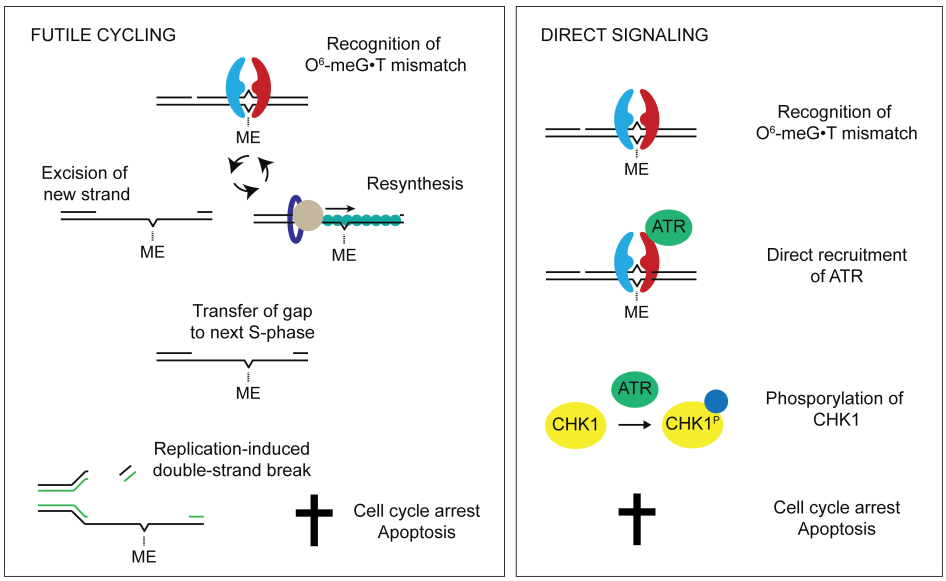


Figure 3. *Possible mechanisms of the MMR-mediated response to methylating agents. Left panel: MutSα binds the mismatched, methylated basepair and triggers excision. Since the methylated nucleotide is in the parental strand, it is not excised and gap-filling creates a novel MMR substrate, which induces rounds of futile cycling. Gapped intermediates are transferred to the next cell cycle, in which they are replicated and generate a double-strand break, which triggers apoptosis. Right panel: MutSα binds the mismatched, methylated basepair and directly recruits ATR, which phosphorylates its target CHK1, leading to cell cycle arrest and apoptosis.*

Cancer in Mammals

The accumulation of mutations in DNA is proposed to be a driving force for cancer development [Hanahan and Weinberg, 2000, 2011], and this is reflected in mouse models of MMR. These models have directly linked loss of MMR to cancer predisposition, and help to understand the etiology and tissue distribution of tumours (reviewed in [Wei et al., 2002; Edelmann and Edelmann, 2004; Taketo and Edelmann, 2009]).

In terms of cancer predisposition, the divergent phenotypes of *Msh2*$^{-/-}$, *Msh3*$^{-/-}$ and *Msh6*$^{-/-}$ mice reflect the functional redundancy between MutSα and MutSβ. *Msh2*$^{-/-}$ mice die within a year due to strong cancer predisposition [de Wind et al., 1995, 1999]. The majority of these mice develop T-cell lymphomas. *Msh6*$^{-/-}$ mice are also tumor prone, but less than their *Msh2*$^{-/-}$ counterparts, as 75% of mice succumb within a year [Edelmann et al., 1997; de Wind et al., 1999]. *Msh3*$^{-/-}$ mice have no apparent phenotype, but in an *Msh6*$^{-/-}$ background, Msh3 loss accelerates tumorigenesis [de Wind et al., 1999].

Interestingly, inactivation of either *Mlh1* or *Pms2* leads to infertility [Baker et al., 1996; Edelmann et al., 1996]. Mlh1-deficient spermatocytes exhibit high levels of prematurely separated chromosomes and arrest in the first division of meiosis, suggesting involvement of Mlh1 in meiotic crossing over. *Mlh1*$^{-/-}$ animals are, like the *Msh2*$^{-/-}$ mice, highly cancer prone. *Pms2*$^{-/-}$ mice succumb to cancer later in life, suggesting functional redundancy of

Pms2 with other heterodimeric Mlh1 partners [Prolla et al., 1998]. Mouse knockouts of the exonuclease involved in MMR, Exo1, are predisposed to cancer but not as strong as *Msh2*$^{-/-}$ or *Mlh1*$^{-/-}$ mice. As mentioned before, this suggests that other exonucleases or mechanisms may substitute for Exo1 function [Wei et al., 2003].

The mouse and human MMR systems are very similar. This is reflected by the fact that the human MLH1 cDNA is able to complement MMR defects in mouse *Mlh1*$^{-/-}$ fibroblasts [Buermeyer et al., 1999]. While MMR deficient mice rapidly develop cancer, mice heterozygous for an MMR gene do not. Such mice are MMR proficient and have to stochastically lose their remaining wild type allele through Loss of Heterozygosity (LOH), and subsequently accumulate mutations for mutation-driven carcinogenesis. Due to the relatively short lifespan of mice, there likely is insufficient time for these events. In contrast, humans heterozygous for an MMR gene suffer from a cancer susceptibility, called Lynch syndrome.

Lynch Syndrome

Lynch syndrome (LS) is a hereditary cancer predisposition syndrome characterized by an increased risk of cancers, mainly of the gastrointestinal and genitourinary tracts (reviewed in [Lynch and de la Chapelle, 2003; Lagerstedt Robinson et al., 2007; Boland and Lynch, 2013]). LS is caused by inherited heterozygous mutations in one of the MMR genes (see below). Since the acquisition of a single affected allele through the germline is considered to cause LS, it is inherited in an autosomal dominant fashion. However, since LS patients require a second hit at a cellular level to induce a phenotype, LS is considered recessive cellularly. It is the stochastic loss of the second, wild type, allele (Loss of Heterozygosity, LOH) that inactivates MMR in a subset of cells. This leads to an accumulation of mutations and the accelerated onset of cancer.

Genetics of Lynch Syndrome

Lynch syndrome is typically caused by mutations in *MSH2, MLH1*, *MSH6* or *PMS2* [Fishel et al., 1993; Leach et al., 1993; Bronner et al., 1994; Nicolaides et al., 1994; Edelmann et al., 1997]. Mutations in *MLH1* and *MSH2* account for the majority of Lynch syndrome (42% and 33%, respectively; [Plazzer et al., 2013]). This is in line with the genetics of MMR, where *MSH2* and *MLH1* are major players in the pathway. *MSH6* and *PMS2* are less important players in LS due to their redundancy with other MMR factors, and cover 18 and 7% of the MMR gene mutations, respectively [Gruber, 2006, Plazzer et al., 2013]. This distribution of mutations is probably subject to an ascertainment bias, as historically, highly penetrant families have been diagnosed first, leading to an overrepresentation of mutations in *MSH2* and *MLH1*.

Besides the four core MMR genes, other genes such as *MLH3* and *EXO1* have also been implicated in LS, but their roles are less clear or controversial [Wu et al., 2001b; Peltomäki and Vasen, 2004]. Some *MLH3* mutations are found in families with atypical LS features [Liu et al., 2003], while other studies were unable to identify a correlation between LS and *MLH3* [Lipkin et al., 2000]. *EXO1* mutations were also found in families with atypical LS [Wu et al., 2001a].

In the last few years, evidence for epimutations as a cause of LS has emerged. The *MSH2* gene can be inactivated *in cis* due to deletions of the 3' region of its upstream gene *TACSTD1*, which

encodes the EpCAM protein. In tissues expressing the mutated *TACSTD1* gene, transcription extends into *MSH2*, thereby resulting in methylation and silencing of the *MSH2* promoter [Ligtenberg et al., 2009]. Carriers of such a mutation have an LS-like phenotype since they are heterozygous for an MMR gene-inactivating mutation. However, since MMR deficiency is limited to EpCAM expressing tissues, their tumor spectrum tends to be different (Table 1). As for *MLH1*, Mendelian inheritance of a constitutional *MLH1* epimutation was shown to be induced by a c.-27C>A nucleotide variant within the 5' UTR [Hitchins et al., 2011].

Characteristics of Lynch Syndrome

Lynch syndrome is noteworthy for its tumor spectrum, including cancers of the gastrointestinal tract such as colorectal, stomach, pancreas, biliary tract and small bowel cancer, but also endometrial, ovarian, ureter/renal pelvis, brain and/or sebaceous gland cancers. The risk of developing such tumors depends on both the tumor type as well as the affected gene (Table 1; [Stoffel et al., 2009; Bonadona et al., 2011; Engel et al., 2012]). The age of onset of colorectal cancer (CRC) for LS patients is on average 45 years old (versus 63 years in the general population).

A hallmark of MMR deficient cancer is MSI, which is found in more than 80% of all Lynch syndrome-related tumors [de la Chapelle and Hampel, 2010]. MSI is typically found in tumors deficient for MSH2 or MLH1. MSH6 deficient tumors typically show lower levels of MSI, due to the functional redundancy of MutSα with MutSβ. Though most microsatellites are found within noncoding regions, some are found within tumor-suppressor genes such as *TGFβRII* and *IGF2R*, involved in growth, *BAX*, involved in apoptosis, and the DNA repair genes *BLM*, *MRE11*, *RAD50*, *MSH3* and *MSH6* [Giráldez et al., 2008]. *TGFβRII* for example, is inactivated in ~80% of MMR-deficient tumors [Markowitz et al., 1995;

Table 1. Risk at Lynch syndrome-related tumors per genotype, as decribed in several studies.

	Cumulative Risk, % (95% Conf. Interval)[a]				
	Gene				
Cancer subtype	MLH1	MSH2	MSH6	PMS2	EPCAM
Colorectal	41 (25-70) [b]	48 (30-77) [b]	12 (8-22) [b]	20 (11-34) [d]	75 (65-85) [c]
	79 (68-90) [c]	77 (64-90) [c]	50 (38-62) [c]		
Endometrial	54 (20-80) [b] 33 (15-51) [c]	21 (8-77) [b] 51 (33-69) [c]	16 (8-32) [b] 34 (20-48) [c]	15 (6-35) [d]	12 (0-27) [c]
Ovarian	20 (1-65) [b]	24 (3-52) [b]	1 (0-3) [b]	NA	NA
Stomach	6 (0.2-17) [b]	0.2 (0-10) [b]	0 [b]	NA	NA
Urothelium	0.2 (0-2.6) [b]	2.2 (0.6-8) [b]	0.7 (0-2.1) [b]	NA	NA
Small bowel	0.4 (0.1-3) [b]	1.1 (0-5) [b]	0 [b]	NA	NA
Biliary tract	1.9 (0-15) [b]	0.02 (0-0.2) [b]	0 [b]	NA	NA

NA: No data available. [a] Cumulative cancer risk at 70 years of Lynch syndrome associated cancers according to the mutated gene, [b] [Bonadona et al., 2011], [c] [Kempers et al., 2011], [d] [Senter et al., 2008].

Alhopuro et al., 2012]. MSI in these genes may be a bystander effect of MMR deficiency, but may also contribute to carcinogenesis. The MSI phenotype is also considered typical for the development of LS tumors, which are usually (near) diploid [Boland and Goel, 2010; de la Chapelle and Hampel, 2010]. This is in contrast with most sporadic CRCs that are aneuploid, and consistent with the hypothesis that MMR-deficient tumors are not driven by gross chromosomal instability (called the CIN phenotype; [Hanahan and Weinberg, 2000, 2011]) but by their intrinsic mutator phenotype.

In contrast to the knockout mice LS patients do not develop lymphomas, and develop tumors only in a subset of tissues. This seems a paradox, since MMR is active in all dividing cells. Several properties of MMR-deficient cells, together with the microenvironment of the colon, may explain the tumor tropism in LS patients [Chao and Lipkin, 2006; Hofstra et al., 2008]. First, the exposure to mutagens from bile and food in the proximal colon [Medina-Arana et al., 2012] may increase the mutation or LOH rate of the remaining wild type MMR allele in cells heterozygous for an MMR gene [Borgdorff et al., 2005]. In light of this, LS (or MSI) tumors are often found in the right-sided (proximal) colon, while CIN tumors are found more distally/left sided [Jass, 2004]. This can be explained by the mutagenicity of products secreted by the gallbladder, which is proximal of the colon. Second, MMR deficient cells are tolerant to various DNA damaging agents, giving these cells a survival advantage over neighboring cells that allows their clonal outgrowth [Toft et al., 1999]. In addition, the mutator phenotype of these MMR deficient cells is boosted by the hypermutability to the mutagens they are exposed to [Glaab et al., 1998; Toft et al., 1999]. The high proliferation rate of the epithelial cells of the colon provides an opportunity for mutations to accumulate. All of these factors may contribute to the tumor spectrum of LS patients [Kloor et al., 2012].

Diagnosis of Lynch Syndrome

Recognition of genetic susceptibility in suspected LS patients is critical for the clinical management of these patients [Vasen et. al, 2013]. LS patients comply to one of a specified set of criteria named the Revised Bethesda Criteria (Figure 4). These criteria are:

- CRC before 50 years of age.
- Presence of synchronous, metachronous colorectal or another LS-associated tumor (colorectal, endometrial, stomach, ovarian, pancreas, ureter/renal pelvis, biliary tract, small bowel, brain and/or sebaceous gland), regardless of age.
- CRC with MSI-H histology in a patient below 60 years of age.
- CRC diagnosed in one or more first-degree relatives with an LS-related tumor, one of which is diagnosed before the age of 50.
- CRC diagnosed in two or more first or second-degree relatives with LS-related tumors, regardless of age.

Once an individual complies to one of these criteria, that individual is clinically diagnosed as an LS patient. Even though the Revised Bethesda criteria are still commonly used, they lack sensitivity to diagnose less penetrant alleles, especially in genes such as *MSH6* and

PMS2 [Sjursen et al., 2010; Moreira et al., 2012]. Besides the Besthesda criteria, alternative prediction models for MMR gene mutations have been described but these seem to perform similarly [Win et. al, 2013]. Several studies have shown that sensitivity can be increased by screening all CRC cases (independent of the Bethesda criteria) for MMR gene mutations, but this is not yet cost-effective [Hampel et al., 2005; Moreira et al., 2012].

Diagnosis of LS is completed only by pinpointing a genetic defect in an MMR gene in these patients, therefore, a clinical diagnosis *per se* does not formally confirm the syndrome. Even so, once LS is clinically diagnosed in the index patient, the index patient and all direct relatives enroll in interval screening procedures. These include colonoscopies every one to two years, to be able to detect colorectal carcinomas or their precursor lesions at an early stage. Such surveillance effectively reduces morbidity and mortality [Lagerstedt Robinson et al., 2007]. In addition, annual gynecological examination, vaginal ultrasound and testing for the tumor biomarker CA-125 in blood is recommended for women, due to the increased risk of endometrial carcinomas [Chen et al., 2007]. It is only a genetic diagnosis which enables the presymptomatic diagnosis of LS, and will enable the differentiation between family members with normal or increased cancer risk. As a consequence, unaffected relatives can be liberated from the burden associated with screening.

Genetic testing in suspected LS patients is a stepwise process, which starts by testing the proband for MMR gene mutations. This is usually preceded by prescreening methods such as MSI testing and immunohistochemistry (IHC; Figure 4). As a pre-screening before genetic testing, nuclear expression of MMR proteins in tumors is visualized by antibody staining. IHC has the advantage that it indicates the MMR gene most eligible for sequencing analysis. The absence of expression of one of the MMR proteins is suggestive for a germline mutation within its corresponding gene and will indicate its sequencing. Only the loss of MSH6 or PMS2 can be visualized by itself, whereas loss MSH2 is always accompanied by MSH6 loss, and MLH1 loss is always accompanied by PMS2 loss [Hendriks et al., 2006]. This feature has been attributed to the loss of protein stability due to loss of dimer formation [de Wind et al., 1999; Hendriks et al., 2006]. Therefore, loss of either MSH6 or PMS2 implies a mutation in its respective gene, loss of both MSH2 and MSH6 implies an MSH2 gene defect and loss of both MLH1 and PMS2 implies an MLH1 defect (Figure 4). IHC is considered a superior predictor of MMR gene mutations, with a sensitivity of 88-90% and a specificity of 84-99% [Niessen et al., 2006].

In case IHC is inconclusive, MSI is considered a good secondary selection criterion for MMR gene mutation screening, as MSI is seen in most of the LS-associated tumors [de la Chapelle and Hampel, 2010]. The sensitivity of MSI for an MMR gene mutation is 82% and the specificity is 70% [Niessen et al., 2006]. MSI is typically tested by PCR-amplifying five microsatellites: mononucleotide repeats BAT-25 and BAT-26 and dinucleotide repeats D2S123, D5S346 and D17S250 [Umar et al., 2004; Boland and Goel, 2010; de la Chapelle and Hampel, 2010]. Recently, though, a panel of the mononucleotide repeats BAT-25, BAT-26, NR-21, NR-24 and NR-27 was put forward as an alternative, as testing for dinucleotide repeats is relatively insensitive [Goel et al., 2010]. The size of these microsatellites is assessed after PCR amplification and fragment analysis.

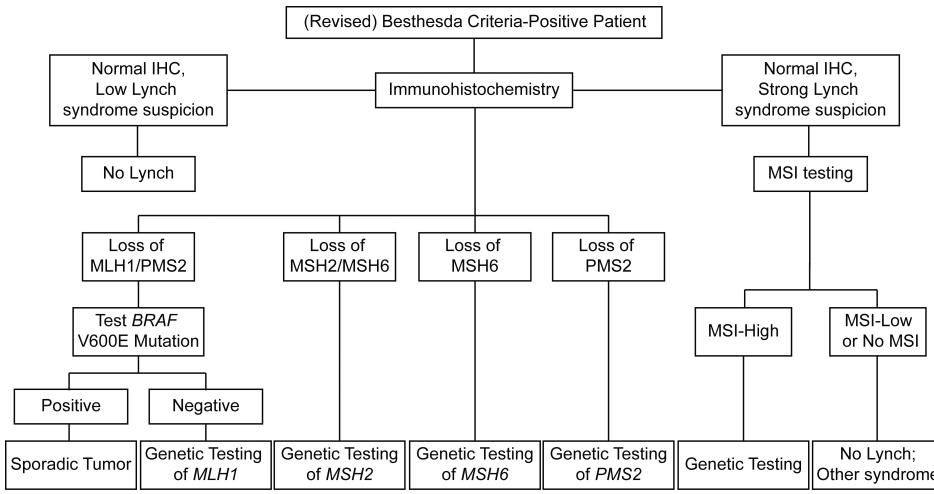


***Figure 4**. The Lynch syndrome diagnosic tree. Adapted from [Coetzee et al., 2013].*

Since not only LS tumors, but also 10-15% of all sporadic tumors express MSI, MSI is not highly specific for LS. MSI in sporadic cases is mostly a consequence of hypermethylation of the 5'CpG island of the *MLH1* promoter, leading to transcriptional silencing [Herman et al., 1998]. To screen against such tumors, MSI testing is often accompanied by testing for the *BRAF* V600E mutation. The presence of this mutation is associated with *MLH1* silencing but not with LS, therefore, wild type *BRAF* together with MSI suggests a germline MMR defect (Figure 4; [Domingo et al., 2005]).

Implications of Lynch Syndrome Diagnosis

As described, confirmed LS patients benefit from tight surveillance programs. In addition, LS patients benefit from chemoprevention, such as daily aspirin intake which significantly reduces the incidence of cancer [Burn et al., 2011]. Both smoking and a high body-mass index (BMI) increase CRC risk in LS patients [van Duijnhoven et al., 2013]. Even though consumption of red meat and alcohol increase the risk of sporadic CRC, such an association has not been shown for LS patients [van Duijnhoven et al., 2013].

LS patients benefit from stratified therapy [Hewish et al., 2010]. For instance, CRC is treated with adjuvant chemotherapy that includes 5-FU, amongst others [Devaud and Gallinger, 2013]. Unfortunately, 5-FU only improves survival of microsatellite-stable tumors and not of MSI tumors [Ribic et al., 2003], in agreement with functional data that shows that MMR-deficient cells are tolerant to 5-FU [Meyers et al., 2003; Liu et al., 2008]. Therefore, LS patients do not benefit from standard treatment regimens. Novel approaches to treat MMR deficient cancers are mostly based on synthetic lethality. Silencing of PTEN-induced putative kinase 1 (PINK1) [Martin et al., 2011] or polymerase β (POLβ) [Martin et al., 2010] is synthetically lethal with MMR deficiency, as is treatment with the drug methotrexate [Martin

et al., 2009]. All of these treatments lead to an increase in oxidative stress, suggesting that oxidative DNA-damaging agents may be utilized to selectively target MMR-deficient cancers [Brierley and Martin, 2013]. Of note, methotrexate induces amplification of the *DHFR* locus and of the adjacent *MSH3*. This increases MSH3 protein levels, which sequesters MSH2 into MutSβ and decreases MutSα levels, impairing MMR [Marra et al., 1998; Chang et al., 2002]. Methotrexate selectively retards MMR deficient (tumor) cells, but as an adverse effect may also induce MMR deficiency in normal somatic cells. In addition, the high levels of oxidative stress in these cells may be toxic, but could also further boost the mutator phenotype in the absence of MMR. Additional (mouse) experiments are required to validate the therapeutic potential of PINK1/POLß silencing and/or methotrexate treatment.

In addition to the mentioned therapies, MMR-deficient tumors may be treated by a selective gene therapy. This method is based on combining a harmless prodrug with a gene encoding a suicide enzyme. The gene is out-of-frame, resulting from a microsatellite within the gene. Only in MMR-deficient (tumor) cells the microsatellite size decreases, the suicide gene is rendered in frame and the suicide enzyme is produced, converting the prodrug into a toxic metabolite that kills the cells [Ferrás et al., 2009].

Variants of Uncertain Significance

Once a variant MMR allele has been identified, its relevance to the disease phenotype needs to be determined. In several classes of mutations, such as deletions, frameshifts or truncating mutations, this is achieved relatively easy. These mutant proteins usually lose their function and/or are degraded by the proteasome. For mutations that do not lead to protein truncations, such as silent mutations, missense mutations, intron variants or small in-frame insertion/deletions, classification is not straightforward. These variants are often referred to as Variants of Uncertain Significance (VUS). The lack of classification of these VUS precludes diagnosis for carriers and their relatives. Therefore, when a suspected Lynch syndrome patient carries a VUS, all first-degree relatives currently enroll lifelong periodic screening, irrespective of their mutation status [Castells et al., 2009]. Due to the inability to diagnose such variants, genetic testing is often not offered to the proband's relatives. The uncertainty of their disease status and the screening itself pose a physical and psychological burden on these families, but also a burden on the preventive health care apparatus.

Currently, 34% of all variants in MMR genes comprise missense mutations [Plazzer et al., 2013]. Inactivating missense mutations may currently remain under detected. The current LS diagnosis protocol only implies an MMR gene for genetic testing once loss of expression of the respective protein is shown in IHC, even though inactivating missense mutations do not necessarily destabilize a protein [Drost et al., 2013]. Now that DNA sequencing technologies are becoming cheaper and more widely applied, the stringency at which MMR genes are selected for sequencing may lower, and the incidence of MMR gene VUS is believed to further increase [Heinen, 2010; Rasmussen et al., 2012]. The development and use of robust and accessible approaches to identify pathogenic VUS will enable the implementation of targeted preventive and curative healthcare for carriers of pathogenic

MMR gene VUS. Meanwhile, unaffected relatives of such carriers can be liberated from the burden associated with the uncertain pathogenicity of the VUS while unwarranted inflow into preventive healthcare systems is reduced [Hennink et al., 2013].

Classification of VUS

VUS may be classified using a large set of criteria, such as:

- Segregation of the VUS with disease within affected families,
- *De novo* appearance of a VUS,
- Presence of the VUS in individuals with a second, obviously pathogenic, MMR gene defect arguing against the pathogenicity of the VUS,
- Absence of the VUS in control sets,
- Change in amino acid polarity or size,
- Presence of the VUS at a predicted splice site.

Unfortunately, such criteria cannot always be applied, since family- or segregation data are often absent, or families are too small to calculate segregation. Even if such correlations can be made, they do not provide definitive proof of pathogenicity. Segregation of a VUS, for example, only suggests that the allele segregates with disease. Therefore, additional genetic factors on that allele, other than the VUS, may cause cancer predisposition. Definite proof of pathogenicity can be provided by assays that test the functional activity of the VUS.

AIM OF THIS THESIS

The inability to assess the pathogenicity of VUS in DNA Mismatch Repair genes is a bottleneck in LS diagnosis. Lack of pathogenic assessment precludes diagnosis for carriers and their relatives, precludes targeted preventive and curative healthcare for carriers of pathogenic VUS and clutters preventive healthcare systems. For these reasons, there is a need for convenient functional assays for the diagnostic assessment of MMR gene VUS. This thesis focuses on the development such assays, while making use of the well-established genetics and biochemistry of the MMR pathway.

The thesis starts with a discussion of recent advances in functional analysis of Variants of Uncertain Significance in DNA Mismatch Repair genes (**Chapter 2;** [Rasmussen et al., 2012]). In **Chapter 3**, I describe the development of a functional assay based on measuring MMR activity of *MSH2* and *MSH6* VUS *in vitro* that allows for a rapid assessment of pathogenicity [Drost et al., 2012]. A similar assay is applied for VUS in *MLH1* [Drost et al., 2010] and *PMS2*, which is discussed in **Chapters 4** and **5**, respectively. **Chapter 6** describes a different approach for the functional analysis of MMR gene VUS. Here, a genetic screen is developed and experimentally validated that systematically identifies residues essential for *in vivo* function of *Msh2* [Drost et al., 2013]. Finally, in **Chapter 7** a similar genetic screen is applied to *Msh6*, and the generated cell lines are used to study mismatch binding and intramolecular signaling in MutSα.

REFERENCES

Alhopuro P, Sammalkorpi H, Niittymäki I, Biström M, Raitila A, Saharinen J, Nousiainen K, Lehtonen HJ, Heliövaara E, Puhakka J, Tuupanen S, Sousa S et al. 2012. Candidate driver genes in microsatellite-unstable colorectal cancer. Int J Cancer 130: 1558-1566.

Antony E, Hingorani MM. 2003. Mismatch recognition-coupled stabilization of Msh2-Msh6 in an ATP-bound state at the initiation of DNA repair. Biochemistry 42: 7682-7693.

Antony E, Khubchandani S, Chen S, Hingorani MM. 2006. Contribution of Msh2 and Msh6 subunits to the asymmetric ATPase and DNA mismatch binding activities of Saccharomyces cerevisiae Msh2-Msh6 mismatch repair protein. DNA Repair 5: 153-162.

Arana ME, Kunkel TA. 2010. Mutator phenotypes due to DNA replication infidelity. Semin Cancer Biol 20: 304-311.

Baker SM, Plug AW, Prolla TA, Bronner CE, Harris AC, Yao X, Christie DM, Monell C, Arnheim N, Bradley A, Ashley T, Liskay RM. 1996. Involvement of mouse Mlh1 in DNA mismatch repair and meiotic crossing over. Nat Genet 13: 336-342.

Boland CR, Goel A. 2010. Microsatellite instability in colorectal cancer. Gastroenterology 138: 2073-2087.e3.

Boland CR, Lynch HT. 2013. The History of Lynch Syndrome. Fam Cancer 12:147-157.

Bonadona V, Bonaïti B, Olschwang S, Grandjouan S, Huiart L, Longy M, Guimbaud R, Buecher B, Bignon YJ, Caron O, Colas C, Noguès C et al. 2011. Cancer risks associated with germline mutations in MLH1, MSH2, and MSH6 genes in Lynch syndrome. JAMA 305: 2304-2310.

Borgdorff V, van Hees-Stuivenberg S, Meijers CM, de Wind N. 2005. Spontaneous and mutagen-induced loss of DNA mismatch repair in Msh2-heterozygous mammalian cells. Mutat Res 574: 50-57.

Brierley DJ, Martin SA. 2013. Oxidative stress and the DNA mismatch repair pathway. Antioxid Redox Signal 18: 2420-2428.

Bronner CE, Baker SM, Morrison PT, Warren G, Smith LG, Lescoe MK, Kane M, Earabino C, Lipford J, Lindblom A. 1994. Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. Nature 368: 258-261.

Buermeyer AB, Wilson-Van Patten C, Baker SM, Liskay RM. 1999. The human MLH1 cDNA complements DNA mismatch repair defects in Mlh1-deficient mouse embryonic fibroblasts. Cancer Res 59: 538-541.

Burn J, Gerdes AM, Macrae F, Mecklin JP, Moeslein G, Olschwang S, Eccles D, Evans DG, Maher ER, Bertario L, Bisgaard ML, Dunlop MG et al. 2011. Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: an analysis from the CAPP2 randomised controlled trial. Lancet 378: 2081-2087.

Cannavo E, Marra G, Sabates-Bellver J, Menigatti M, Lipin SM, Fischer F, Cejka P, Jiricny J. 2005. Expression of the MutL Homologue hMLH3 in Human Cells and its Role in DNA Mismatch Repair. Cancer Res 65: 10759-10766

Castells A, Castellví-Bel S, Balaguer F. 2009. Concepts in familial colorectal cancer: where do we stand and what is the future? Gastroenterology 137: 404-409.

Cejka P, Mojas N, Gillet L, Schär P, Jiricny J. 2005. Homologous recombination rescues mismatch-repair-dependent cytotoxicity of S(N)1-type methylating agents in S. cerevisiae. Curr Biol 15: 1395-1400.

Chang CL, Marra G, Chauhan DP, Ha HT, Chang DK, Ricciardiello L, Randolph A, Carethers JM, Boland CR. 2002. Oxidative stress inactivates the human DNA mismatch repair system. Am J Physiol, Cell Physiol 283: C148-54.

Chao EC, Lipkin SM. 2006. Molecular models for the tissue specificity of DNA mismatch repair-deficient carcinogenesis. Nucleic Acids Res 34: 840-852.

Chen LM, Yang KY, Little SE, Cheung MK, Caughey AB. 2007. Gynecologic cancer prevention in Lynch syndrome/hereditary nonpolyposis colorectal cancer families. Obstet Gynecol 110: 18-25.

Ciccia A, Elledge SJ. 2010. The DNA damage response: making it safe to play with knives. Mol Cell 40: 179-204.

Coetzee E, Algar U, Goldberg P. 2013. New Insights into Lynch Syndrome Diagnosis. DNA Alterations in Lynch Syndrome (Book).

Constantin N, Dzantiev L, Kadyrov FA, Modrich PL. 2005. Human mismatch repair: reconstitution of a nick-directed bidirectional reaction. J Biol Chem 280: 39752-39761.

Cox EC, Degnen GE, Scheppe ML. 1972. Mutator gene studies in Escherichia coli: the mutS gene. Genetics 72: 551-567.

de la Chapelle A, Hampel H. 2010. Clinical relevance of microsatellite instability in colorectal cancer. J Clin Oncol 28: 3380-3387.

de Wind N, Dekker M, Berns A, Radman M, te Riele H. 1995. Inactivation of the mouse Msh2 gene results in mismatch repair deficiency, methylation tolerance, hyperrecombination, and predisposition to cancer. Cell 82: 321-330.

de Wind N, Dekker M, Claij N, Jansen L, van Klink Y, Radman M, Riggins G, van der Valk M, van't Wout K, te Riele H. 1999. HNPCC-like cancer predisposition in mice through simultaneous loss of Msh3 and Msh6 mismatch-repair protein functions. Nat Genet 23: 359-362.

Deans AJ, West SC. 2011. DNA interstrand crosslink repair and cancer. Nat Rev Cancer 11: 467-480.

Debiak M, Nikolova T, Kaina B. 2004. Loss of ATM sensitizes against O6-methylguanine triggered apoptosis, SCEs and chromosomal aberrations. DNA Repair 3: 359-368.

Devaud N, Gallinger S. 2013. Chemotherapy of MMR-deficient colorectal cancer. Fam Cancer 12: 301-306.

Domingo E, Niessen RC, Oliveira C, Alhopuro P, Moutinho C, Espín E, Armengol M, Sijmons RH, Kleibeuker JH, Seruca R, Aaltonen LA, Imai K et al. 2005. BRAF-V600E is not involved in the colorectal tumorigenesis of HNPCC in patients with functional MLH1 and MSH2 genes. Oncogene 24: 3995-3998.

Drost M, Lutzen A, Van Hees S, Ferreira D, Calleja F, Zonneveld J, Nielsen F, Rasmussen L, de Wind N. 2013. Genetic screens to identify pathogenic gene variants in the common cancer predisposition Lynch syndrome. Proc Natl Acad Sci USA 110: 9403-9408.

Drost M, Zonneveld JB, Van Dijk L, Morreau H, Tops CM, Vasen HFA, Wijnen J, De Wind N. 2010. A cell-free assay for the functional analysis of variants of the mismatch repair protein MLH1. Hum Mutat 31: 247-253.

Drost M, Zonneveld JB, van Hees S, Rasmussen LJ, Hofstra RM, De Wind N. 2012. A rapid and cell-free assay to test the activity of lynch syndrome-associated MSH2 and MSH6 missense variants. Hum Mutat 33: 488-494.

Duckett DR, Drummond JT, Murchie AI, Reardon JT, Sancar A, Lilley DM, Modrich P. 1996. Human MutSalpha recognizes damaged DNA base pairs containing O6-methylguanine, O4-methylthymine, or the cisplatin-d(GpG) adduct. Proc Natl Acad Sci USA 93: 6443-6447.

Dzantiev L, Constantin N, Genschel J, Iyer RR, Burgers PM, Modrich PL. 2004. A defined human system that supports bidirectional mismatch-provoked excision. Mol Cell 15: 31-41.

Edelmann L, Edelmann W. 2004. Loss of DNA mismatch repair function and cancer predisposition in the mouse: animal models for human hereditary nonpolyposis colorectal cancer. American journal of medical genetics Part C, Seminars in medical genetics 129: 91-99.

Edelmann W, Cohen PE, Kane M, Lau K, Morrow B, Bennett S, Umar A, Kunkel T, Cattoretti G, Chaganti R, Pollard JW, Kolodner RD et al. 1996. Meiotic pachytene arrest in MLH1-deficient mice. Cell 85: 1125-1134.

Edelmann W, Yang K, Umar A, Heyer J, Lau K, Fan K, Liedtke W, Cohen PE, Kane MF, Lipford JR, Yu N, Crouse GF et al. 1997. Mutation in the mismatch repair gene Msh6 causes cancer susceptibility. Cell 91: 467-477.

Engel C, Loeffler M, Steinke V, Rahner N, Holinski-Feder E, Dietmaier W, Schackert HK, Goergens H, von Knebel Doeberitz M, Goecke TO, Schmiegel W, Buettner R et al. 2012. Risks of less common cancers in proven mutation carriers with lynch syndrome. J Clin Oncol 30: 4409-4415.

Ferrás C, Oude Vrielink JA, Verspuy JW, te Riele H, Tsaalbi-Shtylik A, de Wind N. 2009. Abrogation of Microsatellite-instable Tumors Using a Highly Selective Suicide Gene/Prodrug Combination. Mol Ther 17: 1373-1380

Fishel R, Lescoe MK, Rao MR, Copeland NG, Jenkins NA, Garber J, Kane M, Kolodner R. 1993. The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer. Cell 75: 1027-1038.

Genschel J, Modrich P. 2009. Functions of MutLalpha, replication protein A (RPA), and HMGB1 in 5'-directed mismatch repair. J Biol Chem 284: 21536-21544.

Ghodgaonkar MM, Lazzaro F, Olivera-Pimentel M, Artola-Borán M, Cejka P, Reijns MA, Jackson AP, Plevani P, Muzi-Falconi M, Jiricny J. 2013. Ribonucleotides misincorporated into DNA act as strand-discrimination signals in eukaryotic mismatch repair. Mol Cell 50: 323-332.

Giráldez MD, Castellví-Bel S, Balaguer F, Gonzalo V, Ocaña T, Castells A. 2008. Lynch syndrome in colorectal cancer patients. Expert Rev Anticancer Ther 8: 573-583.

Glaab WE, Risinger JI, Umar A, Barrett JC, Kunkel TA, Tindall KR. 1998. Resistance to 6-thioguanine in mismatch repair-deficient human cancer cell lines

correlates with an increase in induced mutations at the HPRT locus. Carcinogenesis 19: 1931-1937.

Goel A, Nagasaka T, Hamelin R, Boland CR. 2010. An optimized pentaplex PCR for detecting DNA mismatch repair-deficient colorectal cancers. PLoS ONE 5: e9393.

Goldmacher VS, Cuzick RA, Thilly WG. 1986. Isolation and partial characterization of human cell mutants differing in sensitivity to killing and mutation by methylnitrosourea and N-methyl-N'-nitro-N-nitrosoguanidine. J Biol Chem 261: 12462-12471.

Gorman J, Chowdhury A, Surtees JA, Shimada J. 2007. Dynamic Basis for One-Dimensional DNA Scanning by the Mismatch Repair Complex Msh2-Msh6. Mol Cell 28: 359-370

Gradia S, Subramanian D, Wilson T, Acharya S, Makhov A, Griffith J, Fishel R. 1999. hMSH2-hMSH6 forms a hydrolysis-independent sliding clamp on mismatched DNA. Mol Cell 3: 255-261.

Gruber S. 2006. New Developments in Lynch Syndrome (Hereditary Nonpolyposis Colorectal Cancer) and Mismatch Repair Gene Testing. Gastroenterology 130: 577-587.

Gueneau E, Dherin C, Legrand P, Tellier-Lebegue C, Gilquin B, Bonnesoeur P, Londino F, Quemener C, Le Du MH, Márquez JA, Moutiez M, Gondry M et al. 2013. Structure of the MutLα C-terminal domain reveals how Mlh1 contributes to Pms1 endonuclease site. Nat Struct Mol Biol 20: 461-468.

Gupta S, Gellert M, Yang W. 2012. Mechanism of mismatch recognition revealed by human MutSβ bound to unpaired DNA loops. Nat Struct Mol Biol 19: 72-78.

Hampel H, Frankel WL, Martin E, Arnold M, Khanduja K, Kuebler P, Nakagawa H, Sotamaa K, Prior TW, Westman J, Panescu J, Fix D et al. 2005. Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer). N Engl J Med 352: 1851-1860.

Hanahan D, Weinberg RA. 2000. The hallmarks of cancer. Cell 100: 57-70.

Hanahan D, Weinberg RA. 2011. Hallmarks of cancer: the next generation. Cell 144: 646-674.

Hays JB, Hoffman PD, Wang H. 2005. Discrimination and versatility in mismatch repair. DNA Repair 4: 1463-1474.

Heinen CD. 2010. Genotype to phenotype: analyzing the effects of inherited mutations in colorectal cancer families. Mutat Res 693: 32-45.

Hendriks Y, De Jong A, Morreau H, Tops CM, Vasen HFA, Wijnen J, Breuning M, Brocker-Vriends A. 2006. Diagnostic Approach and Management of Lynch Syndrome (Hereditary Nonpolyposis Colorectal Carcinoma): A Guide for Clinicians. CA: A Cancer Journal for Clinicians 56: 213-225.

Hennink SD, Hofland N, Gopie JP, van der Kaa C, de Koning K, Nielsen M, Tops C, Morreau H, de Vos Tot Nederveen Cappel WH, Langers AM, Hardwick JC, Gaarenstroom KN et al. 2013. Value-based healthcare in Lynch syndrome. Fam Cancer 12: 347-354.

Herman JG, Umar A, Polyak K, Graff JR, Ahuja N, Issa JP, Markowitz S, Willson JK, Hamilton SR, Kinzler KW, Kane MF, Kolodner RD et al. 1998. Incidence and functional consequences of hMLH1 promoter hypermethylation in colorectal carcinoma. Proc Natl Acad Sci USA 95: 6870-6875.

Hewish M, Lord CJ, Martin SA, Cunningham D, Ashworth A. 2010. Mismatch repair deficient colorectal cancer in the era of personalized treatment. Nat Rev Clin Oncol 7: 197-208.

Hitchins MP, Rapkins RW, Kwok CT, Srivastava S, Wong JJ, Khachigian LM, Polly P, Goldblatt J, Ward RL. 2011. Dominantly inherited constitutional epigenetic silencing of MLH1 in a cancer-affected family is linked to a single nucleotide variant within the 5'UTR. Cancer Cell 20: 200-213.

Hoeijmakers JH. 2009. DNA damage, aging, and cancer. N Engl J Med 361: 1475-1485.

Hoffman PD, Wang H, Lawrence CW, Iwai S, Hanaoka F, Hays JB. 2005. Binding of MutS mismatch repair protein to DNA containing UV photoproducts, "mismatched" opposite Watson--Crick and novel nucleotides, in different DNA sequence contexts. DNA Repair 4: 983-993.

Hofstra RM, Spurdle AB, Eccles D, Foulkes WD, De Wind N, Hoogerbrugge N, Hogervorst FB, Group IARCUGVW. 2008. Tumor characteristics as an analytic tool for classifying genetic variants of uncertain clinical significance. Hum Mutat 29: 1292-1303.

Holland IB, Blight MA. 1999. ABC-ATPases, adaptable energy generators fuelling transmembrane movement of a variety of molecules in organisms from bacteria to humans. J Mol Biol 293: 381-399.

Hombauer H, Campbell C, Smith C, Desai A, Kolodner R. 2011a. Visualization of Eukaryotic DNA Mismatch Repair Reveals Distinct Recognition and Repair Intermediates. Cell 147: 1040-1053.

Hombauer H, Srivatsan A, Putnam C, Kolodner R. 2011b. Mismatch Repair, But Not Heteroduplex Rejection, Is Temporally Coupled to DNA Replication. Science 334: 1713-1716.

Hsieh P, Yamane K. 2008. DNA mismatch repair: molecular mechanism, cancer, and ageing. Mech Ageing Dev 129: 391-407.

Iyer RR, Pluciennik A, Burdett V, Modrich PL. 2006. DNA mismatch repair: functions and mechanisms. Chem Rev 106: 302-323.

Iyer RR, Pohlhaus TJ, Chen S, Hura GL, Dzantiev L, Beese LS, Modrich PL. 2008. The MutSalpha-proliferating cell nuclear antigen interaction in human DNA mismatch repair. J Biol Chem 283: 13310-13319.

Jackson SP, Bartek J. 2009. The DNA-damage response in human biology and disease. Nature 461: 1071-1078.

Jass JR. 2004. Role of the pathologist in the diagnosis of hereditary non-polyposis colorectal cancer. Dis Markers 20: 215-224.

Jiricny J. 2013. Postreplicative mismatch repair. Cold Spring Harbor Perspectives in Biology 5.

Jiricny J, Su SS, Wood SG, Modrich P. 1988. Mismatch-containing oligonucleotide duplexes bound by the E. coli mutS-encoded protein. Nucleic Acids Res 16: 7843-7853.

Kadyrov FA, Dzantiev L, Constantin N, Modrich PL. 2006. Endonucleolytic function of MutLalpha in human mismatch repair. Cell 126: 297-308.

Kadyrov FA, Genschel J, Fang Y, Penland E, Edelmann W, Modrich P. 2009. A possible mechanism for exonuclease 1-independent eukaryotic mismatch repair. Proc Natl Acad Sci USA 106: 8495-8500.

Kaina B, Christmann M, Naumann S, Roos WP. 2007. MGMT: key node in the battle against genotoxicity, carcinogenicity and apoptosis induced by alkylating agents. DNA Repair 6: 1079-1099.

Kat A, Thilly WG, Fang WH, Longley MJ, Li GM, Modrich P. 1993. An alkylation-tolerant, mutator human cell line is deficient in strand-specific mismatch repair. Proc Natl Acad Sci USA 90: 6424-6428.

Kempers MJ, Kuiper RP, Ockeloen CW, Chappuis PO, Hutter P, Rahner N, Schackert HK, Steinke V, Holinski-Feder E, Morak M, Kloor M, Büttner R et al. 2011. Risk of colorectal and endometrial cancers in EPCAM deletion-positive Lynch syndrome: a cohort study. Lancet Oncol 12: 49-55.

Klapacz J, Meira LB, Luchetti DG, Calvo JA, Bronson RT, Edelmann W, Samson LD. 2009. O6-methylguanine-induced cell death involves exonuclease 1 as well as DNA mismatch recognition in vivo. Proc Natl Acad Sci USA 106: 576-581

Kloor M, Huth C, Voigt AY, Benner A, Schirmacher P, von Knebel Doeberitz M, Bläker H. 2012. Prevalence of mismatch repair-deficient crypt foci in Lynch syndrome: a pathological study. Lancet Oncol 13: 598-606.

Korhonen MK, Vuorenmaa E, Nyström M. 2008. The first functional study of MLH3 mutations found in cancer patients. Genes Chromosomes Cancer 47: 803-809.

Labazi M, Jaafar L, Flores-Rozas H. 2009. Modulation of the DNA-binding activity of Saccharomyces cerevisiae MSH2-MSH6 complex by the high-mobility group protein NHP6A, in vitro. Nucleic Acids Res 37 :7581-7589

Lagerstedt Robinson K, Liu T, Vandrovcova J, Halvarsson B, Clendenning M, Frebourg T, Papadopoulos N, Kinzler KW, Vogelstein B, Peltomäki P, Kolodner RD, Nilbert M et al. 2007. Lynch syndrome (hereditary nonpolyposis colorectal cancer) diagnostics. J Natl Cancer Inst 99: 291-299.

Lahue RS, Au KG, Modrich PL. 1989. DNA mismatch correction in a defined system. Science 245: 160-164.

Lamers MH, Perrakis A, Enzlin JH, Winterwerp HH, de Wind N, Sixma TK. 2000. The crystal structure of DNA mismatch repair protein MutS binding to a G x T mismatch. Nature 407: 711-717.

Leach FS, Nicolaides NC, Papadopoulos N, Liu B, Jen J, Parsons R, Peltomäki P, Sistonen P, Aaltonen LA, Nyström-Lahti M. 1993. Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. Cell 75: 1215-1225.

Li F, Mao G, Tong D, Huang J, Gu L, Yang W, Li GM. 2013. The Histone Mark H3K36me3 Regulates Human DNA Mismatch Repair through Its Interaction with MutSα. Cell 153: 590-600.

Li GM. 2008. Mechanisms and functions of DNA mismatch repair. Cell Res 18: 85-98.

Ligtenberg MJ, Kuiper RP, Chan TL, Goossens M, Hebeda KM, Voorendt M, Lee TY, Bodmer D, Hoenselaar E, Hendriks-Cornelissen SJ, Tsui WY, Kong CK et al. 2009. Heritable somatic methylation and inactivation of MSH2 in families with Lynch syndrome due to deletion of the 3' exons of TACSTD1. Nat Genet 41: 112-117.

Lipkin SM, Wang V, Jacoby R, Banerjee-Basu S, Baxevanis AD, Lynch HT, Elliott RM, Collins FS. 2000. MLH3: a DNA mismatch repair gene associated with mammalian microsatellite instability. Nat Genet 24: 27-35.

Liu A, Yoshioka K, Salerno V, Hsieh P. 2008. The mismatch repair-mediated cell cycle

checkpoint response to fluorodeoxyuridine. J Cell Biol 105: 245-254.

Liu HX, Zhou XL, Liu T, Werelius B, Lindmark G, Dahl N, Lindblom A. 2003. The role of hMLH3 in familial colorectal cancer. Cancer Res 63: 1894-1899.

Liu Y, Fang Y, Shao H, Lindsey-Boltz L, Sancar A, Modrich P. 2009. Interactions of human mismatch repair proteins MutS and MutL with proteins of the ATR-Chk1 pathway. J Biol Chem 285: 5974-5982

Liu Y, Kadyrov FA, Modrich P. 2011. PARP-1 enhances the mismatch-dependence of 5'-directed excision in human mismatch repair in vitro. DNA Repair 10: 1145-1153.

Longley MJ, Pierce AJ, Modrich PL. 1997. DNA Polymerase δ Is Required for Human Mismatch Repair in Vitro. J Biol Chem 272: 10917-10921

Lujan SA, Williams JS, Clausen AR, Clark AB, Kunkel TA. 2013. Ribonucleotides are signals for mismatch repair of leading-strand replication errors. Mol Cell 50: 437-443.

Lynch HT, de la Chapelle A. 2003. Hereditary colorectal cancer. N Engl J Med 348: 919-932.

Macpherson P, Barone F, Maga G, Mazzei F, Karran P, Bignami M. 2005. 8-oxoguanine incorporation into DNA repeats in vitro and mismatch recognition by MutSalpha. Nucleic Acids Res 33: 5094-5105.

Markowitz S, Wang JY, Myeroff L, Parsons R, Sun L, Lutterbaugh J, Fan RS, Zborowska E, Kinzler KW, Vogelstein B. 1995. Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. Science 268: 1336-1338.

Marra G, Iaccarino I, Lettieri T, Roscilli G, Delmastro P, Jiricny J. 1998. Mismatch repair deficiency associated with overexpression of the MSH3 gene. Proc Natl Acad Sci USA 95: 8568-8573.

Martin SA, Hewish M, Sims D, Lord CJ, Ashworth A. 2011. Parallel high-throughput RNA interference screens identify PINK1 as a potential therapeutic target for the treatment of DNA mismatch repair-deficient cancers. Cancer Res 71: 1836-1848.

Martin SA, Mccabe N, Mullarkey M, Cummins R, Burgess DJ, Nakabeppu Y, Oka S, Kay E, Lord CJ, Ashworth A. 2010. DNA polymerases as potential therapeutic targets for cancers deficient in the DNA mismatch repair proteins MSH2 or MLH1. Cancer Cell 17: 235-248.

Martin SA, McCarthy A, Barber LJ, Burgess DJ, Parry S, Lord CJ, Ashworth A. 2009. Methotrexate induces oxidative DNA damage and is selectively lethal to tumour cells with defects in the DNA mismatch repair gene MSH2. EMBO Mol Med 1: 323-337.

Mazurek A, Berardini M, Fishel R. 2002. Activation of human MutS homologs by 8-oxo-guanine DNA damage. J Biol Chem 277: 8260-8266.

McMurray CT. 2010. Mechanisms of trinucleotide repeat instability during human development. Nat Rev Genet 11: 786-799.

Medina-Arana V, Delgado L, Bravo A, Martín J, Fernández-Peralta AM, González-Aguilera JJ. 2012. Tumor spectrum in lynch syndrome, DNA mismatch repair system and endogenous carcinogens. J Surg Oncol 106: 10-16.

Meyers M, Hwang A, Wagner MW, Bruening AJ, Veigl ML, Sedwick WD, Boothman DA. 2003. A role for DNA mismatch repair in sensing and responding to fluoropyrimidine damage. Oncogene 22: 7376-7388.

Mojas N, Lopes M, Jiricny J. 2007. Mismatch repair-dependent processing of methylation damage gives rise to persistent single-stranded gaps in newly replicated DNA. Genes Dev 21: 3342-3355.

Moreira L, Balaguer F, Lindor N, de la Chapelle A, Hampel H, Aaltonen LA, Hopper JL, Le Marchand L, Gallinger S, Newcomb P, Haile R, Thibodeau SN et al. 2012. Identification of Lynch syndrome among patients with colorectal cancer. JAMA 308: 1555-1565.

Nicolaides NC, Papadopoulos N, Liu B, Wei YF, Carter KC, Ruben SM, Rosen CA, Haseltine WA, Fleischmann RD, Fraser CM. 1994. Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. Nature 371: 75-80.

Niessen RC, Berends MJ, Wu Y, Sijmons RH, Hollema H, Ligtenberg MJ, de Walle HE, de Vries EG, Karrenbeld A, Buys CH, van der Zee AG, Hofstra RM et al. 2006. Identification of mismatch repair gene mutations in young patients with colorectal cancer and in patients with multiple tumours associated with hereditary non-polyposis colorectal cancer. Gut 55: 1781-1788.

Obmolova G, Ban C, Hsieh P, Yang W. 2000. Crystal structures of mismatch repair protein MutS and its complex with a substrate DNA. Nature 407: 703-710.

Pabla N, Ma Z, Mcilhatton M, Fishel R, Dong Z. 2011. hMSH2 Recruits ATR to DNA Damage Sites for Activation during DNA Damage-induced Apoptosis. J Biol Chem 286: 10411-10418.

Pavlov YI, Mian IM, Kunkel TA. 2003. Evidence for preferential mismatch repair of lagging strand DNA replication errors in yeast. Curr Biol 13: 744-748.

Peltomäki P, Vasen HFA. 2004. Mutations associated with HNPCC predisposition -- Update of ICG-HNPCC/INSiGHT mutation database. Dis Markers 20: 269-276.

Peña-Diaz J, Jiricny J. 2010. PCNA and MutLα: partners in crime in triplet repeat expansion? Proc Natl Acad Sci USA 107: 16409-16410.

Plazzer JP, Sijmons RH, Woods MO, Peltomäki P, Thompson B, Den Dunnen JT, Macrae F. 2013. The InSiGHT database: utilizing 100 years of insights into Lynch Syndrome. Fam Cancer 12: 175-180.

Pluciennik A, Dzantiev L, Iyer RR, Constantin N, Kadyrov FA, Modrich P. 2010. PCNA function in the activation and strand direction of MutLα endonuclease in mismatch repair. Proc Natl Acad Sci USA 107: 16066-16071.

Prolla TA, Baker SM, Harris AC, Tsao JL, Yao X, Bronner CE, Zheng B, Gordon M, Reneker J, Arnheim N, Shibata D, Bradley A et al. 1998. Tumour susceptibility and spontaneous mutation in mice deficient in Mlh1, Pms1 and Pms2 DNA mismatch repair. Nat Genet 18: 276-279.

Raevaara TE, Korhonen MK, Lohi H, Hampel H, Lynch E, Lönnqvist KE, Holinski-Feder E, Sutter C, McKinnon W, Duraisamy S, Gerdes AM, Peltomäki P et al. 2005. Functional significance and clinical phenotype of nontruncating mismatch repair variants of MLH1. Gastroenterology 129: 537-549.

Rasmussen LJ, Heinen CD, Royer-Pokora B, Drost M, Tavtigian S, Hofstra RM, De Wind N. 2012. Pathological assessment of mismatch repair gene variants in Lynch syndrome: Past, present, and future. Hum Mutat 33: 1617-1625.

Rasmussen LJ, Samson LD. 1996. The Escherichia coli MutS DNA mismatch binding protein specifically binds O(6)-methylguanine DNA lesions. Carcinogenesis 17: 2085-2088.

Ribic CM, Sargent DJ, Moore MJ, Thibodeau SN, French AJ, Goldberg RM, Hamilton SR, Laurent-Puig P, Gryfe R, Shepherd LE, Tu D, Redston M et al. 2003. Tumor microsatellite-instability status as a predictor of benefit from fluorouracil-based adjuvant chemotherapy for colon cancer. N Engl J Med 349: 247-257.

Sacho EJ, Kadyrov FA, Modrich PL, Kunkel TA, Erie DA. 2008. Direct Visualization of Asymmetric Adenine Nucleotide-Induced Conformational Changes in MutLalpha. Mol Cell 29: 112-121.

Schaetzlein S, Chahwan R, Avdievich E, Roa S, Wei K, Eoff RL, Sellers RS, Clark AB, Kunkel TA, Scharff MD, Edelmann W. 2013. Mammalian Exo1 encodes both structural and catalytic functions that play distinct roles in essential biological processes. Proc Natl Acad Sci USA 110: E2470-9.

Senter L, Clendenning M, Sotamaa K, Hampel H, Green J, Potter JD, Lindblom A, Lagerstedt K, Thibodeau SN, Lindor NM, Young J, Winship I et al. 2008. The clinical phenotype of Lynch syndrome due to germ-line PMS2 mutations. Gastroenterology 135: 419-428.

Sjursen W, Haukanes BI, Grindedal EM, Aarset H, Stormorken A, Engebretsen LF, Jonsrud C, Bjørnevoll I, Andresen PA, Ariansen S, Lavik LA, Gilde B et al. 2010. Current clinical criteria for Lynch syndrome are not sensitive enough to identify MSH6 mutation carriers. J Med Genet 47: 579-585.

Stoffel E, Mukherjee B, Raymond VM, Tayob N, Kastrinos F, Sparr J, Wang F, Bandipalliam P, Syngal S, Gruber SB. 2009. Calculation of Risk of Colorectal and Endometrial Cancer Among Patients With Lynch Syndrome. Gastroenterology 137: 1621-1627

Stojic L, Brun R, Jiricny J. 2004. Mismatch repair and DNA damage signalling. DNA Repair 3: 1091-1101.

Stojic L, Cejka P, Jiricny J. 2005. High doses of SN1 type methylating agents activate DNA damage signaling cascades that are largely independent of mismatch repair. Cell Cycle 4: 473-477.

Stojic L, Mojas N, Cejka P, di Pietro M, Ferrari S, Jiricny J. 2004. Mismatch repair-dependent G2 checkpoint induced by low doses of SN1 type methylating agents requires the ATR kinase. Genes Dev 18: 1331-1344.

Strand M, Prolla TA, Liskay RM, Petes TD. 1993. Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair. Nature 365: 274-276.

Swann PF, Waters TR, Moulton DC, Xu YZ, Zheng Q, Edwards M, Mace R. 1996. Role of postreplicative DNA mismatch repair in the cytotoxic action of thioguanine. Science 273: 1109-1111.

Taketo MM, Edelmann W. 2009. Mouse models of colon cancer. Gastroenterology 136: 780-798.

Toft NJ, Winton DJ, Kelly J, Howard LA, Dekker M, te Riele H, Arends MJ, Wyllie AH, Margison GP, Clarke AR. 1999. Msh2 status modulates both apoptosis and mutation frequency in the murine small intestine. Proc Natl Acad Sci USA 96: 3911-3915.

Umar A, Risinger JI, Hawk ET, Barrett JC. 2004. Testing guidelines for hereditary non-polyposis colorectal cancer. Nat Rev Cancer 4: 153-158.

van Duijnhoven FJ, Botma A, Winkels R, Nagengast FM, Vasen HF, Kampman E. 2013. Do lifestyle factors influence colorectal cancer risk in Lynch syndrome? Fam Cancer 12: 285-293.

Vasen HF, Blanco I, Aktan-Collan K, Gopie JP, Alonso A, Aretz S, Bernstein I, Bertario L, Burn J, Capella G, Colas C, Engel C et al. 2013. Revised guidelines for the clinical management of Lynch syndrome (HNPCC): recommendations by a group of European experts. Gut 62: 812–823

Warren JJ, Forsberg LJ, Beese LS. 2006. The structural basis for the mutagenicity of O(6)-methyl-guanine lesions. Proc Natl Acad Sci USA 103: 19701-19706.

Warren JJ, Pohlhaus TJ, Changela A, Iyer RR, Modrich PL, Beese LS. 2007. Structure of the human MutSalpha DNA lesion recognition complex. Mol Cell 26: 579-592.

Wei K, Clark AB, Wong E, Kane MF, Mazur DJ, Parris T, Kolas NK, Russell R, Hou H, Kneitz B, Yang G, Kunkel TA et al. 2003. Inactivation of Exonuclease 1 in mice results in DNA mismatch repair defects, increased cancer susceptibility, and male and female sterility. Genes Dev 17: 603-614.

Wei K, Kucherlapati R, Edelmann W. 2002. Mouse models for human DNA mismatch-repair gene defects. Trends in molecular medicine 8: 346-353.

Win AK, Macinnis RJ, Dowty JG, Jenkins MA. 2013. Criteria and prediction models for mismatch repair gene mutations: a review. J Med Genet 50:785–793

Wu Y, Berends MJ, Post JG, Mensink RG, Verlind E, Van Der Sluis T, Kempinga C, Sijmons RH, van der Zee AG, Hollema H, Kleibeuker JH, Buys CH et al. 2001a. Germline mutations of EXO1 gene in patients with hereditary nonpolyposis colorectal cancer (HNPCC) and atypical HNPCC forms. Gastroenterology 120: 1580-1587.

Wu Y, Berends MJ, Sijmons RH, Mensink RG, Verlind E, Kooi KA, Van Der Sluis T, Kempinga C, van Der Zee AG, Hollema H, Buys CH, Kleibeuker JH et al. 2001b. A role for MLH3 in hereditary nonpolyposis colorectal cancer. Nat Genet 29: 137-138.

Yamane K, Schupp JE, Kinsella TJ. 2007. BRCA1 activates a G2-M cell cycle checkpoint following 6-thioguanine-induced DNA mismatch damage. Cancer Res 67: 6286-6292.

York SJ, Modrich PL. 2006. Mismatch Repair-dependent Iterative Excision at Irreparable O6-Methylguanine Lesions in Human Nuclear Extracts. J Biol Chem 281: 22674-22683

Yoshioka K, Yoshioka Y, Hsieh P. 2006. ATR kinase activation mediated by MutSalpha and MutLalpha in response to cytotoxic O6-methylguanine adducts. Mol Cell 22: 501-510.

Yuan F, Gu L, Guo S, Wang C, Li GM. 2004. Evidence for involvement of HMGB1 protein in human DNA mismatch repair. J Biol Chem 279: 20935-20940.

Zhang Y, Yuan F, Wang D, Gu L, Li GM. 2008. Identification of RFX as a novel mismatch repair stimulatory factor. J Biol Chem 283: 12730-12735