

The mechanism of mismatch repair and the functional analysis of mismatch repair defects in Lynch syndrome

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Abstract The majority of Lynch syndrome (LS), also known as hereditary non-polyposis colorectal cancer (HNPCC), has been linked to heterozygous defects in DNA mismatch repair (MMR). MMR is a highly conserved pathway that recognizes and repairs polymerase misincorporation errors and nucleotide damage as well as functioning as a damage sensor that signals apoptosis. Loss-of-heterozygosity (LOH) that retains the mutant MMR allele and epigenetic silencing of MMR genes are associated with an increased mutation rate that drives carcinogenesis as well as microsatellite instability that is a hallmark of LS/HNPCC. Understanding the biophysical functions of the MMR components is crucial to elucidating the role of MMR in human tumorigenesis and determining the pathogenetic consequences of patients that present in the clinic with an uncharacterized variant of the MMR genes. We summarize the historical association between LS/HNPCC and MMR, discuss the mechanism of the MMR and finally examine the functional analysis of MMR defects found in LS/HNPCC patients and their relationship with the severity of the disease.

Keywords DNA mismatch repair system · Lynch syndrome · Hereditary non polyposis colorectal cancer (HNPCC) · Functional defects · hMSH2–hMSH6 · hMLH1–hPMS2

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Introduction

The history of the Lynch syndrome or hereditary nonpolyposis colorectal cancer (LS/HNPCC) dates back to 1895, when Dr. Aldred Warthin, intrigued by an increased onset of bowel and endometrial cancers in the family of his seamstress, performed a complete study of this family that was ultimately published in 1913 [1, 2]. The significance of early onset colon, gastric and endometrial carcinomas in the “Family G” of Warthin, could not be totally appreciated until two other families were described by Lynch [1, 3]. These families did not follow the diagnostic for Familial adenomatous polyposis (FAP) and presented with an absence of colon polyps in addition to other types of cancer, among them endometrial cancer.

Discovery of the genes involved in Lynch syndrome

In 1992, the *APC* gene was excluded as responsible for LS/HNPCC [4]. A year later, instability of simple repeated sequences (microsatellite instability or MSI) was detected in sporadic colorectal cancer (CRC) [5] and MSI was found to be associated with CRC tumors in LS/HNPCC patients [6, 7]. The observation of MSI implicated mismatch repair (MMR) processes that had been previously described in bacteria and yeast [8–10]. The MMR system is primarily responsible for the recognition and repair of nucleotide polymerase misincorporation errors introduced during replication [11–13]. Simple repeat sequences appear particularly prone to polymerase misincorporation errors and the resulting MSI appears to be a litmus for MMR defects [14]. The central players in *Escherichia coli* MMR are MutS, MutL and MutH.

In December of 1993, the human MutS homolog (MSH), *hMSH2*, was identified and associated with LS/HNPCC

[15, 16]. In March of 1994, the human MutL homolog, *hMLH1*, was identified and associated with LS/HNPCC [17, 18]. Subsequently, the *hPMS1* and *hPMS2* (post-meiotic segregation MutL homologs) genes were identified and suggested to be causative of LS/HNPCC [19]. Later genetic analysis of the *hPMS1* gene excluded it as contributor to LS/HNPCC, while the *hMSH6* and *hMLH3* genes were ultimately included as causative genes in LS/HNPCC [20, 21].

Microsatellite instability

Vast majority of the cells deficient in MMR develop a “mutator phenotype” characterized by 10^2 – 10^3 fold increase in the spontaneous mutation rate [5, 22]. Elevated mutation rates affect the entire genome including DNA sequences that contain microsatellite repeats [23, 24]. A number of genes have been identified that include microsatellite sequences within their coding region [25, 26]. MSI in these genes results in altered signaling transduction, apoptosis, DNA repair, transcriptional regulation, protein translocation and modifications, and immune monitoring. For example, intragenic MSI results in inactivation of the *TFGβ-RII* tumor suppressor gene in ~80 % of MMR-defective tumors, while the remaining ~20 % appear to inactivate the *IGFR1I* tumor suppressor via intragenic MSI [27, 28]. Similarly, intragenic MSI also appears to inactivate the apoptosis promoter *BAX* [29].

The presence of high level of MSI (MSI-H) [14] is normally associated within a mutation of the *hMLH1* and *hMSH2* genes [30, 31]. A low level of MSI (MSI-L) [14] appears largely due to mutations in the *hMSH6* gene (10 %), and the *hPMS2* gene (5 %). The etiology of approximately 5 % of MSI tumors remains unknown [32]. More than 95 % of LS/HNPCC tumors show MSI, whereas only 10–15 % of the sporadic colorectal cancers display MSI [14, 30, 33]. Importantly, diagnostic MSI has become

a dependable indicator of MMR defects in human tumors once reliable markers were established [14, 34].

Mismatch repair

The conversion of heteroduplex (mismatched) to homoduplex (nonmismatched) DNA following transformation into *Pneumococcus* began studies of MMR in the early 1970s [35, 36]. In 1975, Wildenberg and Meselson [37] demonstrated that *E. coli* differentially corrected λ DNA containing genetically defined mismatched nucleotides. Shortly thereafter, and based on observations of DNA adenine methylation biases in Okazaki fragments by Marinus [38], Radman and Meselson [39] suggested that *E. coli* MMR could correctly identify a polymerase nucleotide misincorporation error within double-stranded DNA (dsDNA) by uniquely excising a transiently unmethylated newly replicated strand. These seminal studies positioned *E. coli* as the paradigm for MMR where the previously identified mutator genes MutS [40], MutL [41], MutH [42], UvrD [42] and the DNA adenine methylase (Dam) [43] were determined to be required for the process. Interestingly, only MutS and MutL appear to be highly conserved throughout evolution, although there may be functional conservation of the other MMR activities (Table 1).

The principal function of the MMR system is to correct DNA polymerase misincorporation errors that arising during DNA replication [44]. Overall the MMR system increases replication fidelity 100- to 1000-fold [45, 46]. MMR is also involved in ensuring the efficiency and fidelity of both mitotic and meiotic DNA recombination, class-switch recombination and somatic hypermutation of the variable regions of immunoglobulin genes, interstrand DNA cross-link repair, repair of aberrant triple-repeat expansions and in

Table 1 DNA mismatch repair protein functions

<i>E. coli</i>	Yeast	Human	Overall function
MutS	Msh2–Msh3 Msh2–Msh6	hMSH2–hMSH3 hMSH2–hMSH6	Mismatch and small IDLs recognition. ATP-bound sliding clamp formation
MutL	Mlh1–Pms1 Mlh1–Mlh2 Mlh1–Mlh3	hMLH1–hPMS2 hMLH1–hPMS1 hMLH1–hMLH3	Coordinator of the downstream processes after mismatch recognition by MutS. GHRL ATPase. Cryptic endonuclease
MutH	–	–	Nick newly synthesized DNA strand in hemimethylated GATC sites
γ-δ complex	RFC complex	RFC complex	β-clamp loading
beta-clamp	PCNA	PCNA	Connects mismatch repair machinery to the replication fork
ExoI, ExoX	ExoI	hEXO1	5′–3′ DNA excision
RecJ ExoVII	?	?	3′–5′ DNA excision

Not represented: DNA polymerases, SSB protein and DNA ligases, since they are post excision

? Represents unknown similar biochemical functions

responses to DNA damage by participating in S and G2/M phase checkpoints as well as apoptosis [47].

The mechanism of mismatch repair

The DNA mismatch repair system is a bidirectional excision-resynthesis system that is initiated at a *defined* strand scission that is 3'- or 5'- of a mismatch and the excision tract extends to a *nonspecific point* just past the mismatch [12, 13]. The process can be divided into four main steps: (1) Recognition of a mismatch by the MSHs, (2) recruitment of the MLHs by ATP-bound MSHs that then connect the mismatch recognition signal to the distant DNA strand scission where excision begins, (3) excision of the DNA strand containing the wrong nucleotide and (4) resynthesis of the excision gap by the replicative DNA polymerase using the remaining DNA strand as a template. This latter step appears virtually identical to normal replicative DNA synthesis and will not be discussed in detail here. Clearly the unique aspect of MMR is the well-defined and targeted mismatch-dependent DNA strand excision that begins at a defined strand scission and extends to a non-specific point just beyond the mismatch.

The mechanism of MMR excision has been controversial and many of the detailed biophysical steps remain poorly understood. One of the most controversial issues was how the recognition of a mismatch is transmitted to a distant strand scission site along the DNA where the excision step begins. In bacteria, excision is initiated at a GATC site that has been incised (nicked) on the unmethylated strand by the MutH protein [48]. The location of this MutH incision can be several thousand nucleotides distant from the mismatch and on either the 5'- or 3'- side of the mismatch [49, 50]. While not absolutely demonstrated, it appears that the DNA strand scissions used to initiate excision in eukaryotes are likely to be the 3'-leading strand or the 5'-lagging strand ends. Thus the requirement of methylation and a MutH homolog appears to have been eliminated in eukaryotes as well as perhaps every organism except gram-negative enteric bacteria like *E. coli*. Regardless, it is clear that in all MMR systems, mismatch recognition by MSH proteins must be transmitted to a distant DNA strand scission.

Three conceptual mechanisms were developed to envision connecting mismatch binding to a distant strand incision [51]: (1) Static Transactivation [52], (2) Hydrolysis-Dependent Translocation [53, 54] and (3) Molecular Switch Sliding Clamp [55–58]. Static Transactivation suggests that a complex of the MMR proteins is assembled at the mismatch, which then awaits a random DNA looping collision event that brings the incision site in contact with this complex. This “trans-activation” model was largely eliminated by the observation that placing a block between

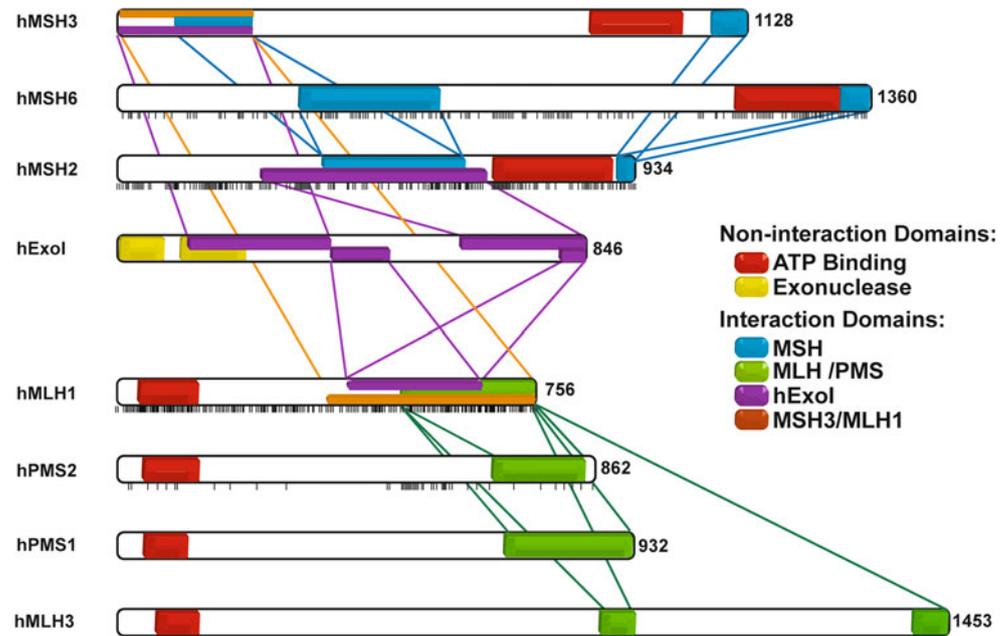
the mismatch and the strand scission blocked MMR [59]. An intervening block should not affect a mechanism where DNA may loop to the distant strand scission site. Yet MMR excision was abolished when a variety of blocks were placed between the mismatch and the strand scission [59].

The remaining two mechanisms may be considered “cis-activation” models since they envision movement of a MMR complex along the DNA helix to the DNA strand scission; where an intervening block would stop MMR. These two models have been very difficult to distinguish since one requires *ATP binding plus hydrolysis* in order to “motor” down the DNA helix (Hydrolysis-Dependent Translocation), while the other only requires mismatch provoked *ATP binding* that results in the formation of an MSH hydrolysis-independent sliding clamp capable of 1-dimensional (1D) diffusion along the DNA helix (Molecular Switch Sliding Clamp). A growing body of evidence, including single molecule analysis, appears to largely underpin the Molecular Switch Sliding Clamp model [60, 61]. Our group developed a complete Molecular Switch Sliding Clamp model for the MMR excision reaction nearly a decade ago [55, 56, 62]. We will highlight several important features of MSH and MLH protein functions within the context of the Molecular Switch Sliding Clamp model where defects may be relevant to the pathobiology of LS/HNPCC.

MutS homologs

All MSH proteins appear to exist as an asymmetric homodimer or heterodimer where the dimeric/heterodimeric interaction domains are confined to the C-terminal regions (Fig. 1; [63]). In addition, MSH proteins are members of the ABC family of ATPases and contain a highly conserved Walker A/B nucleotide binding motif (Fig. 1; [52, 64]). The MSH ATPase domain is absolutely required for translocations from the mismatch to the distant strand scission. A fundamental feature of the Molecular Switch Sliding Clamp model envisions MSH proteins as a mismatch-provoked molecular switch that utilizes nucleotide binding and exchange mechanics similar to G proteins [56]. Most if not all MSH proteins display asymmetric ATP binding by the homodimeric subunits or the heterodimeric partners [65]. In eukaryotes the MSH6 subunit appears to bind and hydrolyze ATP very quickly [66]. The binding of ATP by MSH6 appears to enhance ATP binding by MSH2 [67]. Once the MSH6 subunit hydrolyzes ATP it appears to immediately release the ADP product [66–68]. In contrast, the MSH2 subunit retains the ADP product which appears to suppress further ATP binding by MSH6 [67, 68]. Interaction with a mismatch induces the release of the ADP bound by the MSH2 subunit allowing ATP binding by both

Fig. 1 Interaction regions between MSH, MLH and EXO proteins and distribution of missense mutations in the main MMR proteins. Each vertical bar represents a missense mutation described in a patient and reported to the InSight database. Two important non-interacting regions of these proteins are also represented: the ATP binding domain and the exonuclease domain of hEXO1. Interaction regions among MSH proteins are represented in blue, among MLH/PMS proteins in green and between both groups of proteins in orange. Interactions with hExo1 are represented in purple



MSH2 and MSH6, which results in the formation of a hydrolysis-independent sliding clamp capable of 1D diffusion along the DNA helix [57, 58, 60]. It is this mismatch-provoked nucleotide exchange that identified MSH proteins as a molecular switch [57, 69, 70]. The nature of bacterial MutS has precluded a similarly detailed subunit analysis of ATP bind-hydrolysis and nucleotide exchange. However, bacterial MutS clearly displays mismatch-induced ADP release that results in the formation of an ATP-bound hydrolysis-independent sliding clamp [55, 61]. These observations appear to underline a conserved biophysical mechanism for all MSH proteins [71].

While searching for a mismatch, MSH proteins appear to form an incipient clamp that tracks along the duplex DNA helix and undergoes rotational diffusion while in continuous contact with the backbone [60, 72]. At physiological salt the dwell time (lifetime) of MSH proteins on duplex DNA is approximately 1 s [61]. These same studies determined a diffusion coefficient that suggested a random-walk search distance capable of examining ~700 bp of naked DNA in that time. A diminished search area on chromatin is likely since nucleosomes will introduce a topological barrier to rotational diffusion [73]. In spite of these issues MSH proteins appear capable of recognizing a mismatch within a nucleosome [74].

Once a mismatch is encountered the MSH protein lingers for approximately 3 s [61]. While there have been a number of inferences regarding base-flipping and induced DNA bending [75, 76], it is more likely that MSH proteins linger at the mismatch because they encounter an altered intrinsic flexibility in the DNA surrounding a mismatch [77]. Such backbone flexibility would likely be distinct

from the smooth helical backbone that would promote unhindered rotational diffusion during the mismatch search [77]. The fact that the MSH protein does not detect the mismatch *pre se*, but instead detects the intrinsic DNA flexibility of the mismatch region may explain the wide range of mismatches and lesions recognized by MSH proteins [77]. One imagines that the lingering process provides some time for the ordering of disordered or partially disordered peptides within the MSH dimer/heterodimer [78] that ultimately induces ADP release and ATP binding by both subunits [67, 68].

One of the dimeric bacterial MutS subunits, or in eukaryotes the MSH6 subunit, appears to directly interrogate the mismatched base through a conserved Phe-X-Glu motif in the N-terminal domain during this peptide ordering process [78–80]. The formation of an ATP-bound hydrolysis independent sliding clamp considerably increases the dwell time on the DNA to approximately 10 min and alters the diffusion mechanics such that the MSH freely rotates around the DNA backbone [60, 61]. The altered lifetime and diffusion mechanics suggests MSH proteins may diffuse away from the mismatch by a random-walk that could encompass hundreds of thousands of nucleotides along naked DNA. Moreover, multiple sliding clamps may be loaded once the MSH is released from the mismatch [58]. The long lifetime and multiple sliding clamps results in the ability to displace nucleosomes on model chromatin substrates by occupying transiently unwrapped DNA [81]. One might imagine a similar occupation/clearing mechanism for protein obstacles on DNA surrounding a mismatch that could be important for the MMR excision process.

MutL homologs

The detailed function(s) of the MLH proteins remains enigmatic. All MLH proteins studied to date exist as an asymmetric homodimer or heterodimer where the dimer/heterodimer interaction domains are located at the C-terminus of the respective proteins (Fig. 1; [82]). In addition, MLH proteins contain an N-terminal GHKL ATP binding cassette (Fig. 1; [83]). ATP binding by MLH appears to result in dimerization of the two ATPase-containing N-terminal domains [84, 85]. The role of this dimerization in MMR is unknown. It was reported that MLH proteins bind to single stranded DNA (ssDNA; [86, 87]). However, single molecule analysis has demonstrated that stable ssDNA binding does not occur in physiological salt [88]. These results suggest at best there may be a transient interaction with ssDNA which may have relevance for MMR. Interestingly, the human PMS2 and its yeast homolog Pms1 have been shown to contain an intrinsic endonuclease activity that is located in a largely disordered domain near the C-terminus [89–91]. It is interesting to note that *E. coli* MutL and all known *gram negative* enteric bacterial MLH's do not appear to contain an intrinsic endonuclease, while most other bacteria including *gram positive B. subtilis* MutL do contain an intrinsic endonuclease that is required for MMR [92, 93]. As outlined below, this cryptic endonuclease may account for an apparent lack of a 3'–5' exonuclease in eukaryotes (and perhaps most other types of bacteria) that would be essential to facilitate bidirectional excision surrounding a mismatch.

A large body of evidence suggests that MLH proteins only interact stably with ATP-bound MSH sliding clamps [55, 94–96]. These results strongly suggest that MSH proteins may deliver MLH proteins to the site of excision and/or other functions. There is recent cellular evidence that suggests the MLH proteins may be left behind during or following repair [97]. A lack of clear evidence for MLH functions, once they have interacted with ATP-bound MSH proteins, leaves this part of the MMR mechanism substantially murky. It is likely that direct visualization on single molecules will help to clarify the role of MLH proteins in MMR.

Accessory MMR proteins

Major accessory proteins required for the bacterial MMR excision reaction include the single stranded DNA (ssDNA) binding protein SSB, the RecQ family DNA helicase UvrD, one of four exonucleases (the 5' → 3' exonucleases ExoI and ExoX, or the 3' → 5' exonucleases RecJ and Exo VII), and the replicative processivity factor β -clamp [98–101]. Similarly, the 3' → 5' exonuclease

ExoI, the ssDNA binding heterotrimer RPA and PCNA are major accessory factors in eukaryotic MMR [49, 102, 103]. Interestingly, there have been no helicases shown to be essential for the eukaryotic MMR excision reaction, although there are many more apparently redundant RecQ family helicases in eukaryotes than prokaryotes.

The unique genetic requirement for a single 5' → 3' exonuclease (ExoI) in eukaryotes appeared inconsistent with bidirectional MMR excision until the intrinsic endonuclease in hPMS2 was identified and subsequently verified in *Saccharomyces cerevisiae* Pms1 as well as *B. subtilis* MutL [89, 90, 92, 93]. A simple mechanistic interpretation would suggest that MLH proteins containing the intrinsic endonuclease may occasionally nick the strand being excised in the 3'-direction, and its subsequent thermal or active displacement presents a 5'-end to ExoI for degradation. This result is consistent with biochemical studies that suggest hMLH1–hPMS2 is required for 3'-excision but not 5'-excision [49, 102, 103].

Assessing functional defects associated with MMR proteins

A major issue in the medical genetics of LS/HNPCC is assessing the functional implications of missense MMR gene alterations with unknown significance (Table 2). Such issues do not arise in large families where MMR alterations may be correlated with cancer predisposition. However, the advent of molecular diagnostic technologies where significant family history is either lacking or non-existent has presented genetic counselors and clinicians with diagnostic challenges that may only be resolved with some functional analysis. The problem becomes even more apparent when one localizes the missense variants along the length of the MMR genes (see black ticks below MMR genes in Fig. 1). In the case of both hMSH2 and hMLH1 the missense variants appear to be scattered over the entire length of the genes. This observation appears to preclude a simple bioinformatic analysis that might be based on the

Table 2 Mutations reported in the DNA MMR genes

	hMSH2	hMSH6	hMLH1	hPMS2
Insertions	28 (2)	18 (6)	34 (5)	3 (2)
Deletions	280 (37)	118 (25)	303 (42)	34 (9)
Duplications	74 (7)	57 (18)	778 (4)	8 (1)
Missense	221	161	289	29
Nonsense	107	55	92	18
Splice variants	16	8	51	9

() mutations located in introns, 5' upstream, and 3' downstream of the gene

Source Insight group

identification of important functional domains. Amusingly, for both hMSH6 and hPMS2 there appear to be clusters of mutations that may target important functional regions; although the statistical significance of such an interpretation appears limited by the reduced numbers of variants. Interestingly, an hPMS2 missense mutation cluster appears to be located near the N-terminal side of the hMLH1 interaction domain (Fig. 1). This is the general location of the metal binding domain that is associated with the cryptic endonuclease of hPMS2.

Considering the protein mechanics outlined above one could envision a number of functional defects associated with missense mutation of the MMR genes. Both the MSH and MLH proteins contain heterodimeric interaction domains, essential ATP binding/hydrolysis domains and protein–protein interaction domains that might be susceptible to amino acid changes in the regions (Fig. 1). In addition, there are several important structural features such as the alpha helices in the MSH connector domains that link the mismatch binding domain with the ATP processing domain that might be susceptible to amino acid changes in these regions. It is not surprising that the first attempts at functional analysis focused on the MSH and MLH heterodimer interaction domains; a relatively easy test that used Glutathione-S-Transferase (GST) tagged MMR protein with in vitro transcribed-translated (IVTT) interacting partner [63, 82]. Precipitation of the GST-tagged MMR protein determined whether a missense mutation in the IVTT partner was capable of a stable interaction. Since these early studies functional analysis has fallen into two major categories: (1) targeted functional studies (such as the GST-IVTT interaction analysis), and (2) analysis of the

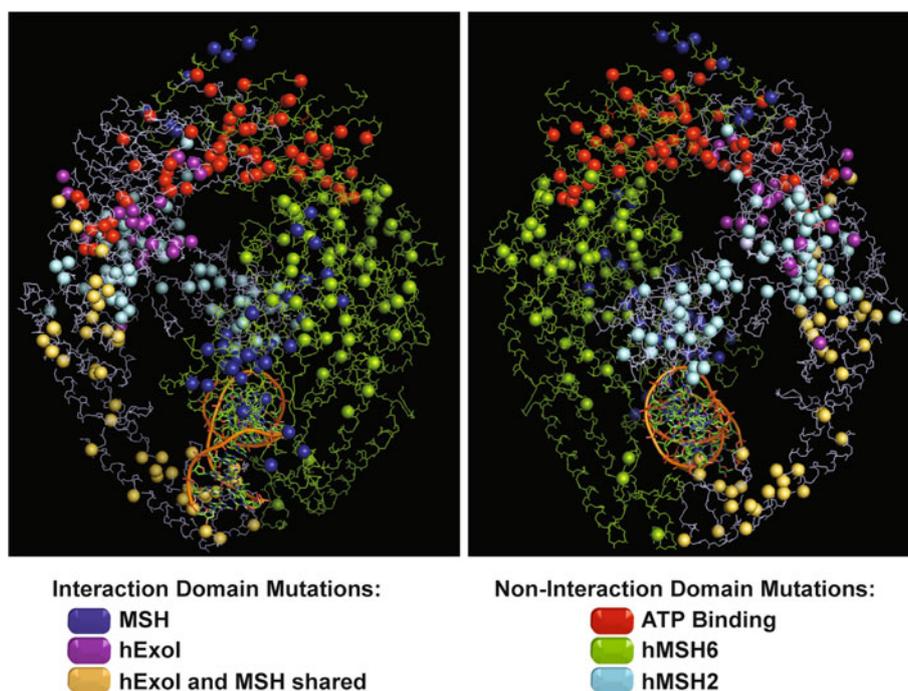
complete MMR reaction. All of the functional analysis assays have both strengths and weaknesses although none could be considered sufficient for a full-proof medical diagnostic.

Targeted functional analysis has identified missense variants that are defective in MMR heterodimer interactions as well as interactions between the MMR proteins [63, 82, 104–108]. These assays included both the GST-IVTT as well as yeast two-hybrid analysis. Immunohistochemical precipitation has provided a potential cellular variation of the interaction assay for hMLH1 that also included studies of protein stability and localization [109]. Combining yeast two hybrid with in vitro mismatch repair assays has similarly extended the interaction analysis [110].

The recently published structure of hMSH2–hMSH6 has allowed the localization of known missense variants of both these proteins (Fig. 2). As with missense mutation localization on the linear protein sequence (Fig. 1), the localization of missense mutations on the 3D structure does not appear to provide additional insights into a useful medical diagnostic (Fig. 2). The biochemical analysis of hMSH2 has examined heterodimer interactions as well as the ability of the protein to process ADP/ATP and form multiple sliding clamps on DNA [63, 111]. It is interesting that several of the ADP/ATP processing functions appear to be altered by missense variants that are distant from the Walker A/B nucleotide binding domain.

Transient MMR protein expression has underpinned the analysis of the complete MMR reaction [112–115]. In these studies the MMR gene is transiently expressed in a complementary defective cell line and the resulting complete

Fig. 2 Spatial distribution of missense mutations described in the hMSH2–hMSH6 protein complex. The hMSH2 and hMSH6 proteins are delineated in *blue* and *green* respectively. Missense mutations are shown as spheres. Mutations located in the ATPase domain are represented in *red*. Mutations located in the interaction domain between both proteins are marked in *dark blue*. Mutations in hMSH2 protein located in the interaction region only with hEXO1 are *purple* and mutations located in the shared interaction region with hEXO1 and hMSH6 are represented in *yellow* (See Fig. 1). Other mutations are colored based on the protein color where it is found



MMR activity is examined. A complete MMR reaction could be determined using one of several *in vitro* repair reactions. A similar cellular extract system may examine potential splice site variants that result in a defective MMR protein [116, 117]. The advantage to all of these assay systems is that if a functional defect is identified, it can often be interpreted as pathogenic. The disadvantage to of the assay systems is the *scale* of significance in any given defect. For example, if a protein interaction is reduced by 10 % is such a defect pathogenic? Moreover, the absence of a defect in any one of these assay systems does not necessarily eliminate a missense variant from being pathological.

Finally, the role of MMR genes in DNA damage sensing may require a completely different type in functional analysis [24, 118, 119]. Perhaps the DNA damage signaling functions of the MMR genes are the crux of tumorigenesis in LS/HNPCC and only a functional assay that examines such will be informative? If this is the case, then none of the functional assays developed to date is sufficient for a clinical diagnostic.

Prospects

Besides the disadvantages of the functional analysis outlined above, a major bottleneck in all of these assays is that each of the missense variants must be constructed *de novo* and then introduced onto the assay system for analysis. For several of the biochemical assays the proteins must be purified to near homogeneity to assure that the analysis is measuring the MMR protein function and not a sample contaminant. In fact, several of the published functional analysis for LS/HNPCC can be easily criticized as measuring indirect activities. Immunoprecipitation assay of MMR interactions would fall into this latter critique since non-specific protein interactions in these complex protein precipitates could affect the outcome. Ideally, one would like to examine a patient sample directly to determine MMR function. In essence this would require an assay that was extremely sensitive and could detect function from very small amounts of tissue samples.

Single molecule analysis may provide both the necessary sensitivity and ability to examine MMR functions with minute samples. As the name suggests, single molecule analysis examines single DNA molecules. Sample sizes are necessarily small and relevant target protein concentrations are usually in the low nanomolar. Moreover, recent studies have demonstrated that complex biological samples may be used in single molecule analysis as long as the read-out is specific [120]. Nearly all single-molecule systems utilize fluorescent probes that either tag the protein or the DNA [121, 122]. Combining two compatible fluorophores allows

the detection of fluorescence resonance energy transfer (FRET), where the emission of one fluorophore may excite a second nearby fluorophore into an emission at a longer wavelength. FRET decays with the sixth power of the distance between fluorophores making it superbly sensitive to very small distance changes. A similar FRET system has been used by our group to examine the biophysical mechanism of MMR [60, 61, 88]. One could easily envision several combined FRET systems that examine mismatch recognition, protein interactions, and perhaps the entire MMR reaction with exceedingly small patient samples. Moreover, the system appears amenable to developing a DNA damage signaling assay that relies on MMR interactions [123].

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Conflict of interest The authors declare no conflicts of interest.

References

1. Lynch HT (1985) Classics in oncology. Aldred Scott Warthin, M.D., Ph.D. (1866–1931). *CA Cancer J Clin* 35(6): 345–7
2. Classics in oncology. Heredity with reference to carcinoma as shown by the study of the cases examined in the pathological laboratory of the University of Michigan, 1895–1913. By Aldred Scott Warthin. 1913. *CA Cancer J Clin* 1985 Nov–Dec; 35(6): 348–59
3. Lynch HT, Smyrk T (1996) Hereditary nonpolyposis colorectal cancer (Lynch syndrome). An updated review. *Cancer* 78(6): 1149–1167
4. Peltomaki P, Sistonen P, Mecklin JP et al (1992) Evidence that the MCC-APC gene region in 5q21 is not the site for susceptibility to hereditary nonpolyposis colorectal carcinoma. *Cancer Res* 52(16):4530–4533
5. Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M (1993) Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. *Nature* 363(6429):558–561
6. Peltomaki P, Lothe RA, Aaltonen LA et al (1993) Microsatellite instability is associated with tumors that characterize the hereditary non-polyposis colorectal carcinoma syndrome. *Cancer Res* 53(24):5853–5855
7. Aaltonen LA, Peltomaki P, Leach FS et al (1993) Clues to the pathogenesis of familial colorectal cancer. *Science* 260(5109): 812–816
8. Levinson G, Gutman GA (1987) High frequencies of short frameshifts in poly-CA/TG tandem repeats borne by bacteriophage M13 in *Escherichia coli* K-12. *Nucleic Acids Res* 15(13):5323–5338
9. Reenan RA, Kolodner RD (1992) Isolation and characterization of two *Saccharomyces cerevisiae* genes encoding homologs of the bacterial HexA and MutS mismatch repair proteins. *Genetics* 132(4):963–973
10. 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(6443):274–276

11. Fishel R, Kolodner RD (1995) Identification of mismatch repair genes and their role in the development of cancer. [Review] [158 refs]. *Curr Opin Genet Dev* 5(3):382–395
12. Kolodner R (1996) Biochemistry and genetics of eukaryotic mismatch repair. [Review] [85 refs]. *Genes Dev* 10(12):1433–1442
13. Modrich P, Lahue R (1996) Mismatch repair in replication fidelity, genetic recombination, and cancer biology. [Review] [225 refs]. *Annu Rev Biochem* 65:101–133
14. Dietmaier W, Wallinger S, Bocker T, Kullmann F, Fishel R, Ruschoff J (1997) Diagnostic microsatellite instability: definition and correlation with mismatch repair protein expression. *Cancer Res* 57(21):4749–4756
15. Fishel R, Lescoe MK, Rao MR et al (1993) The human mutator gene homolog MSH2 and its association with hereditary non-polyposis colon cancer. *Cell* 75(5):1027–1038
16. Leach FS, Nicolaides NC, Papadopoulos N et al (1993) Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer. *Cell* 75(6):1215–1225
17. Bronner CE, Baker SM, Morrison PT et al (1994) Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer. *Nature* 368(6468):258–261
18. Papadopoulos N, Nicolaides NC, Wei YF et al (1994) Mutation of a mutL homolog in hereditary colon cancer. *Science* 263(5153):1625–1629
19. Nicolaides NC, Papadopoulos N, Liu B et al (1994) Mutations of two PMS homologues in hereditary nonpolyposis colon cancer. *Nature* 371(6492):75–80
20. Papadopoulos N, Nicolaides NC, Liu B et al (1995) Mutations of GTBP in genetically unstable cells. *Science* 268:1915–1917
21. Lipkin SM, Wang V, Jacoby R et al (2000) MLH3: a DNA mismatch repair gene associated with mammalian microsatellite instability. *Nat Genet* 24(1):27–35
22. Loeb LA (1991) Mutator phenotype may be required for multistage carcinogenesis. [Review] [63 refs]. *Cancer Res* 51(12):3075–3079
23. Shibata D, Peinado MA, Ionov Y, Malkhosyan S, Perucho M (1994) Genomic instability in repeated sequences is an early somatic event in colorectal tumorigenesis that persists after transformation. *Nat Genet* 6(3):273–281
24. Zhang H, Richards B, Wilson T et al (1999) Apoptosis induced by overexpression of hMSH2 or hMLH1. *Cancer Res* 59(13):3021–3027
25. Duval A, Hamelin R (2002) Genetic instability in human mismatch repair deficient cancers. *Ann Genet* 45(2):71–75
26. Duval A, Hamelin R (2002) Mutations at coding repeat sequences in mismatch repair-deficient human cancers: toward a new concept of target genes for instability. *Cancer Res* 62(9):2447–2454
27. Markowitz S, Wang J, Myeroff L et al (1995) Inactivation of the type II TGF-beta receptor in colon cancer cells with microsatellite instability. *Science* 268(5215):1336–1338
28. Souza RF, Appel R, Yin J et al (1996) Microsatellite instability in the insulin-like growth factor II receptor gene in gastrointestinal tumours. *Nat Genet* 14(3):255–257
29. Rampino N, Yamamoto H, Ionov Y et al (1997) Somatic frameshift mutations in the BAX gene in colon cancers of the microsatellite mutator phenotype. *Science* 275(5302):967–969
30. Boland CR, Thibodeau SN, Hamilton SR et al (1998) A national cancer Institute workshop on microsatellite instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 58(22):5248–5257
31. Frayling IM (1999) Microsatellite instability. *Gut* 45(1):1–4
32. Baudhuin LM, Burgart LJ, Leontovich O, Thibodeau SN (2005) Use of microsatellite instability and immunohistochemistry testing for the identification of individuals at risk for Lynch syndrome. *Fam Cancer* 4(3):255–265
33. Umar A, Boland CR, Terdiman JP et al (2004) Revised Bethesda guidelines for hereditary nonpolyposis colorectal cancer (Lynch syndrome) and microsatellite instability. *J Natl Cancer Inst* 96(4):261–268
34. Bocker T, Diermann J, Friedl W et al (1997) Microsatellite instability analysis: a multicenter study for reliability and quality control. *Cancer Res* 57(21):4739–4743
35. Roger M (1972) Evidence for conversion of heteroduplex transforming DNAs to homoduplex by recipient pneumococcal cells. *Proc Natl Acad Sci USA* 69:466–470
36. Tiraby J-G, Fox MS (1973) Marker discrimination in transformation and mutation of pneumococcus. *Proc Natl Acad Sci USA* 70:3541–3545
37. Wildenberg J, Meselson M (1975) Mismatch repair in heteroduplex DNA. *Proc Natl Acad Sci USA* 72(6):2202–2206
38. Marinus MG (1976) Adenine methylation of Okazaki fragments in *Escherichia coli*. *J Bacteriol* 128(3):853–854
39. Radman M, Wagner RE, Glickman BW, Meselson M (1980) DNA methylation, mismatch correction and genetic stability. In: Alacevic M (ed) *Progress in environmental mutagenesis*. Elsevier/North Holland Biomedical Press, Amsterdam, pp 121–130
40. Siegel EC, Bryson V (1967) Mutator gene of *Escherichia coli* B. *J Bacteriol* 94:38–47
41. Goldstein A, Smoot JS (1955) A strain of *Escherichia coli* with an unusually high rate of auxotrophic mutation. *J Bacteriol* 70:588–595
42. Hill RF (1970) Location of genes controlling excision repair of UV damage and mutator activity in *Escherichia coli* WP2. *Mutat Res* 9(3):341–344
43. Marinus MG (1973) Location of DNA methylation genes on the *Escherichia coli* K-12 genetic map. *Mol Gen Genet* 127(1):47–55
44. Friedberg EC, Walker GC, Siede W, Wood RD, Schultz RA, Ellenberger T (2006) *DNA repair and mutagenesis*, 2nd edn. American Society of Microbiology, Washington
45. Iyer RR, Pluciennik A, Burdett V, Modrich PL (2006) DNA mismatch repair: functions, mechanisms. *Chem Rev* 106(2):302–323
46. Jascur T, Boland CR (2006) Structure and function of the components of the human DNA mismatch repair system. *Int J Cancer* 119(9):2030–2035
47. Jiricny J (2006) The multifaceted mismatch-repair system. *Nat Rev Mol Cell Biol* 7(5):335–346
48. Welsh KM, Lu AL, Clark S, Modrich P (1987) Isolation and characterization of the *Escherichia coli* mutH gene product. *J Biol Chem* 262(32):15624–15629
49. Constantin N, Dzantiev L, Kadyrov FA, Modrich P (2005) Human mismatch repair: reconstitution of a nick-directed bidirectional reaction. *J Biol Chem* 280(48):39752–39761
50. Grilley M, Griffith J, Modrich P (1993) Bidirectional excision in methyl-directed mismatch repair. *J Biol Chem* 268(16):11830–11837
51. Kolodner RD, Mendillo ML, Putnam CD (2007) Coupling distant sites in DNA during DNA mismatch repair. *Proc Natl Acad Sci USA* 104(32):12953–12954
52. Junop MS, Obmolova G, Rausch K, Hsieh P, Yang W (2001) Composite active site of an ABC ATPase: MutS uses ATP to verify mismatch recognition and authorize DNA repair. *Mol Cell* 7(1):1–12
53. Allen DJ, Makhov A, Grilley M et al (1997) MutS mediates heteroduplex loop formation by a translocation mechanism. *EMBO J* 16(14):4467–4476

54. Blackwell LJ, Bjornson KP, Modrich P (1998) DNA-dependent activation of the hMutS alpha ATPase. *J Biol Chem* 273(48):32049–32054
55. Acharya S, Foster PL, Brooks P, Fishel R (2003) The coordinated functions of the *E coli* MutS and MutL proteins in mismatch repair. *Mol Cell* 12(1):233–246
56. Fishel R (1999) Signaling mismatch repair in cancer. *Nat Med* 5(11):1239–1241
57. Gradia S, Acharya S, Fishel R (1997) The human mismatch recognition complex hMSH2–hMSH6 functions as a novel molecular switch. *Cell* 91(7):995–1005
58. Gradia S, Subramanian D, Wilson T et al (1999) hMSH2–hMSH6 forms a hydrolysis-independent sliding clamp on mismatched DNA. *Mol Cell* 3(2):255–261
59. Pluciennik A, Modrich P (2007) Protein roadblocks and helix discontinuities are barriers to the initiation of mismatch repair. *Proc Natl Acad Sci USA* 104(31):12709–12713
60. Cho WK, Jeong C, Kim D et al (2012) ATP alters the diffusion mechanics of MutS on mismatched DNA. *Structure* 20(7):1264–1274
61. Jeong C, Cho WK, Song KM et al (2011) MutS switches between two fundamentally distinct clamps during mismatch repair. *Nat Struct Mol Biol* 18(3):379–385
62. Fishel R, Acharya S, Berardini M et al (2000) Signaling mismatch repair: the mechanics of an adenosine-nucleotide molecular switch. *Cold Spring Harb Symp Quant Biol* 65:217–224
63. Guerrette S, Wilson T, Gradia S, Fishel R (1998) Interactions of human hMSH2 with hMSH3 and hMSH2 with hMSH6: examination of mutations found in hereditary nonpolyposis colorectal cancer. *Mol Cell Biol* 18(11):6616–6623
64. Fishel R, Wilson T (1997) MutS homologs in mammalian cells. [Review] [84 refs]. *Curr Opin Genet Dev* 7(1):105–113
65. Antony E, Hingorani MM (2004) Asymmetric ATP binding and hydrolysis activity of the *Thermus aquaticus* MutS dimer is key to modulation of its interactions with mismatched DNA. *Biochemistry* 43:13115–13128
66. 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 (Amst)* 5(2):153–162
67. Heinen CD, Cyr JL, Cook C et al (2011) Human MSH2 (hMSH2) protein controls ATP processing by hMSH2–hMSH6. *J Biol Chem* 286(46):40287–40295
68. Mazur DJ, Mendillo ML, Kolodner RD (2006) Inhibition of Msh6 ATPase activity by mispaired DNA induces a Msh2(ATP)–Msh6(ATP) state capable of hydrolysis-independent movement along DNA. *Mol Cell* 22(1):39–49
69. Fishel R (1998) Mismatch repair, molecular switches, and signal transduction. [Review] [56 refs]. *Genes Dev* 12(14):2096–2101
70. Gradia S, Acharya S, Fishel R (2000) The role of mismatched nucleotides in activating the hMSH2–hMSH6 molecular switch. *J Biol Chem* 275:3922–3930
71. Snowden T, Acharya S, Butz C, Berardini M, Fishel R (2004) hMSH4–hMSH5 recognizes holliday junctions and forms a meiosis-specific sliding clamp that embraces homologous chromosomes. *Mol Cell* 15(3):437–451
72. Gorman J, Chowdhury A, Surtees JA et al (2007) Dynamic basis for one-dimensional DNA scanning by the mismatch repair complex Msh2–Msh6. *Mol Cell* 28(3):359–370
73. Gorman J, Plys AJ, Visnapuu ML, Alani E, Greene EC (2010) Visualizing one-dimensional diffusion of eukaryotic DNA repair factors along a chromatin lattice. *Nat Struct Mol Biol* 17(8):932–938
74. Li F, Tian L, Gu L, Li GM (2009) Evidence that nucleosomes inhibit mismatch repair in eukaryotic cells. *J Biol Chem* 284(48):33056–33061
75. Kunkel TA, Erie DA (2005) DNA mismatch repair. *Annu Rev Biochem* 74:681–710
76. Sass LE, Lanyi C, Weninger K, Erie DA (2011) Single-molecule FRET TACKLE reveals highly dynamic mismatched DNA–MutS complexes. *Biochemistry* 49(14):3174–3190
77. Mazurek A, Johnson CN, Germann MW, Fishel R (2009) Sequence context effect for hMSH2–hMSH6 mismatch-dependent activation. *Proc Natl Acad Sci USA* 106(11):4177–4182
78. Obmolova G, Ban C, Hsieh P, Yang W (2000) Crystal structures of mismatch repair protein MutS, its complex with a substrate DNA [see comments]. *Nature* 407(6805):703–710
79. 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. [see comments]. *Nature* 407(6805):711–717
80. 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(4):579–592
81. Javaid S, Manohar M, Punja N et al (2009) Nucleosome remodeling by hMSH2–hMSH6. *Mol Cell* 36(6):1086–1094
82. Guerrette S, Acharya S, Fishel R (1999) The interaction of the human MutL homologues in hereditary nonpolyposis colon cancer. *J Biol Chem* 274(10):6336–6341
83. Dutta R, Inouye M (2000) GHKL, An emergent ATPase/kinase superfamily. *Trends Biochem Sci* 25(1):24–28
84. Ban C, Yang W (1998) Crystal structure and ATPase activity of MutL: implications for DNA repair and mutagenesis. *Cell* 95(4):541–552
85. Sacho EJ, Kadyrov FA, Modrich P, Kunkel TA, Erie DA (2008) Direct visualization of asymmetric adenine nucleotide-induced conformational changes in MutLalpha. *Mol Cell* 29(1):112–121
86. Bende SM, Grafstrom RH (1991) The DNA binding properties of the MutL protein isolated from *Escherichia coli*. *Nucleic Acids Res* 19:1549–1555
87. Drotschmann K, Hall MC, Shcherbakova PV et al (2002) DNA binding properties of the yeast Msh2–Msh6, Mlh1–Pms1 heterodimers. *Biol Chem* 383(6):969–975
88. Park J, Jeon Y, In D, Fishel R, Ban C, Lee JB (2010) Single-molecule analysis reveals the kinetics and physiological relevance of MutL–ssDNA binding. *PLoS ONE* 5(11):e15496
89. Kadyrov FA, Dzantiev L, Constantin N, Modrich P (2006) Endonucleolytic function of MutLalpha in human mismatch repair. *Cell* 126(2):297–308
90. Kadyrov FA, Holmes SF, Arana ME et al (2007) *Saccharomyces cerevisiae* MutLalpha is a mismatch repair endonuclease. *J Biol Chem* 282(51):37181–37190
91. Kosinski J, Plotz G, Guarne A, Bujnicki JM, Friedhoff P (2008) The PMS2 subunit of human MutLalpha contains a metal ion binding domain of the iron-dependent repressor protein family. *J Mol Biol* 382(3):610–627
92. Pillon MC, Lorenowicz JJ, Uckelmann M et al (2010) Structure of the endonuclease domain of MutL: unlicensed to cut. *Mol Cell* 39(1):145–151
93. Pillon MC, Miller JH, Guarne A (2010) The endonuclease domain of MutL interacts with the beta sliding clamp. *DNA Repair (Amst)* 10(1):87–93
94. Grilley M, Welsh KM, Su SS, Modrich P (1989) Isolation and characterization of the *Escherichia coli* mutL gene product. *J Biol Chem* 264(2):1000–1004
95. Mendillo ML, Mazur DJ, Kolodner RD (2005) 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* 280(23):22245–22257
96. Schofield MJ, Nayak S, Scott TH, Du C, Hsieh P (2001) Interaction of *Escherichia coli* MutS and MutL at a DNA mismatch. *J Biol Chem* 276(30):28291–28299
97. Hombauer H, Campbell CS, Smith CE, Desai A, Kolodner RD (2011) Visualization of eukaryotic DNA mismatch repair reveals distinct recognition and repair intermediates. *Cell* 147(5):1040–1053
98. Lopez de Saro FJ, Marinus MG, Modrich P, O'Donnell M (2006) The beta sliding clamp binds to multiple sites within MutL and MutS. *J Biol Chem* 281(20):14340–14349
99. Viswanathan M, Lovett ST (1998) Single-strand DNA-specific exonucleases in *Escherichia coli*—roles in repair and mutation avoidance. *Genetics* 149(1):7–16
100. Pluciennik A, Burdett V, Lukianova O, O'Donnell M, Modrich P (2009) Involvement of the beta clamp in methyl-directed mismatch repair in vitro. *J Biol Chem* 284(47):32782–32791
101. Ramilo C, Gu L, Guo S et al (2002) Partial reconstitution of human DNA mismatch repair in vitro: characterization of the role of human replication protein A. *Mol Cell Biol* 22(7):2037–2046
102. Pluciennik A, Dzantiev L, Iyer RR, Constantin N, Kadyrov FA, Modrich P (2010) PCNA function in the activation and strand direction of MutLalpha endonuclease in mismatch repair. *Proc Natl Acad Sci USA* 107(37):16066–16071
103. Zhang Y, Yuan F, Presnell SR et al (2005) Reconstitution of 5'-directed human mismatch repair in a purified system. *Cell* 122(5):693–705
104. Belvederesi L, Bianchi F, Galizia E et al (2008) MSH2 missense mutations, HNPCC syndrome: pathogenicity assessment in a human expression system. *Hum Mutat* 29(11):E296–E309
105. Hardt K, Heick SB, Betz B et al (2011) Missense variants in hMLH1 identified in patients from the German HNPCC consortium, functional studies. *Fam Cancer* 10(2):273–284
106. Kondo E, Suzuki H, Horii A, Fukushige S (2003) A yeast two-hybrid assay provides a simple way to evaluate the vast majority of hMLH1 germ-line mutations. *Cancer Res* 63(12):3302–3308
107. Schmutte C, Marinescu RC, Sadoff MM, Guerrette S, Overhuser J, Fishel R (1998) Human exonuclease I interacts with the mismatch repair protein hMSH2. *Cancer Res* 58(20):4537–4542
108. Schmutte C, Sadoff MM, Shim KS, Acharya S, Fishel R (2001) The interaction of DNA mismatch repair proteins with human exonuclease I. *J Biol Chem* 276(35):33011–33018
109. Raevaara TE, Korhonen MK, Lohi H et al (2005) Functional significance, clinical phenotype of nontruncating mismatch repair variants of MLH1. *Gastroenterology* 129(2):537–549
110. Takahashi M, Shimodaira H, Andreutti-Zaugg C, Iggo R, Kolodner RD, Ishioka C (2007) Functional analysis of human MLH1 variants using yeast and in vitro mismatch repair assays. *Cancer Res* 67(10):4595–4604
111. Heinen CD, Wilson T, Mazurek A, Berardini M, Butz C, Fishel R (2002) HNPCC mutations in hMSH2 result in reduced hMSH2–hMSH6 molecular switch functions. *Cancer Cell* 1:469–478
112. Brieger A, Plotz G, Raedle J et al (2005) Characterization of the nuclear import of human MutLalpha. *Mol Carcinog* 43(1):51–58
113. Lei X, Zhu Y, Tomkinson A, Sun L (2004) Measurement of DNA mismatch repair activity in live cells. *Nucleic Acids Res* 32(12):e100
114. Ollila S, Dermadi Bebek D, Jiricny J, Nystrom M (2008) Mechanisms of pathogenicity in human MSH2 missense mutants. *Hum Mutat* 29(11):1355–1363
115. Trojan J, Zeuzem S, Randolph A et al (2002) Functional analysis of hMLH1 variants, HNPCC-related mutations using a human expression system. *Gastroenterology* 122(1):211–219
116. Naruse H, Ikawa N, Yamaguchi K et al (2009) Determination of splice-site mutations in Lynch syndrome (hereditary non-polyposis colorectal cancer) patients using functional splicing assay. *Fam Cancer* 8(4):509–517
117. Tournier I, Vezain M, Martins A et al (2008) A large fraction of unclassified variants of the mismatch repair genes MLH1, MSH2 is associated with splicing defects. *Hum Mutat* 29(12):1412–1424
118. Fishel R (2001) The selection for mismatch repair defects in hereditary nonpolyposis colorectal cancer: revising the mutator hypothesis. *Cancer Res* 61(20):7369–7374
119. Gong JG, Costanzo A, Yang HQ et al (1999) The tyrosine kinase c-Abl regulates p73 in apoptotic response to cisplatin-induced DNA damage [see comments]. *Nature* 399(6738):806–809
120. Jain A, Liu R, Ramani B et al (2011) Probing cellular protein complexes using single-molecule pull-down. *Nature* 473(7348):484–488
121. Joo C, Balci H, Ishitsuka Y, Buranachai C, Ha T (2008) Advances in single-molecule fluorescence methods for molecular biology. *Annu Rev Biochem* 77:51–76
122. Roy R, Hohng S, Ha T (2008) A practical guide to single-molecule FRET. *Nat Methods* 5(6):507–516
123. Yoshioka K, Yoshioka Y, Hsieh P (2006) ATR kinase activation mediated by MutSalph and MutLalpha in response to cytotoxic O6-methylguanine adducts. *Mol Cell* 22(4):501–510