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A personal historical view of DNA mismatch repair with an emphasis on eukaryotic DNA mismatch repair

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1. The early days of mismatch repair

I first learned about the existence of DNA mismatch repair (MMR) in the early 1970's as a graduate student from Robert "Bob" C. Warner, who was one of the few people in our department whose lab worked on DNA. Bob was interested in isolating and proving the existence of Holliday junction-containing DNA molecules, the homologous recombination (HR) intermediate Robin Holliday had postulated in 1964 to form mispaired bases that could then account for some aspects of gene conversion when they were acted on by MMR [1]. I only later became aware of parallel studies on the possibility that MMR functioned in mutagenesis and replication fidelity [2]; the roles of mismatch repair in suppressing HR between divergent DNA and in the DNA damage response were discovered many years later (Discussed below). My many stimulating discussions with Bob led me to purify the only restriction endonuclease known at the time, the Hind II+III mixture, for use by his lab in their studies of HR intermediates. This experience led me away from my graduate studies with Krishna Tewari with whom I had worked performing quite challenging studies on the molecular characterization of chloroplast DNA to doing postdoctoral work on the enzymology of DNA replication. While I really enjoyed my postdoctoral studies on bacteriophage T7 DNA replication proteins and felt I was learning about protein purification from a true master, Charles Richardson, I also felt this was not the area I wanted to establish my own laboratory in. This rekindled my interest in HR and MMR and Charles was extremely generous to let me spend

part of my time using new methods of site-directed mutagenesis to make mutations that altered restriction endonuclease cleavage sites in plasmid DNAs for use in making DNA substrates that I planned to use to study the genetics and biochemistry of HR and MMR. I cannot emphasize enough that I had three great mentors and how they each helped to educate me and aided in the advancement of my career. In this vein, I am also pleased that so many of my own trainees have gone on to establish their own successful labs.

When I moved to the Dana-Farber Cancer Institute in 1978 to start my own lab, I began studying plasmid recombination and, to a lesser extent, MMR in *Escherichia coli*, areas that I really had never worked in before. Given this, it seems amazing that I was able to obtain an academic position and obtain funding for my lab; I think there is a lesson in my career experiences that I hope current day research institutions and funding agencies will reflect on leading them to adjust their ways to make it easier than presently seems possible for young scientists to go in new directions. During the 1960's, 1970's and early 1980's the genetic basis for the model for *E. coli* methyl-directed MMR was worked out, to a great extent through the efforts of the Meselson, Marinus and Radman labs as well as others [3-8]. This system was thought to act on mispairs that were formed as a result of DNA replication errors as well as on mispairs that were formed in heteroduplex HR intermediates when mutant and wild-type single strands were paired with each other [9, 10]. The key MMR genes including *mutS*, *mutL*, *mutH*, *uvrD* and *dam* were identified through genetic studies [9, 10].

Transient under-methylation of the newly synthesized DNA strand was proposed to be the strand specificity mark during post-replication MMR and some studies suggested that MutS might be involved at the mispair recognition step and that MutL and MutH might be involved in strand discrimination [9-12]. These studies as well as the availability of the replication proteins and exonucleases set the stage for the subsequent reconstitution studies from the Modrich lab [13, 14] and mechanistic studies of individual *E. coli* MMR proteins from the Modrich lab and many other labs (For representative reviews see [15, 16]). In retrospect, it is ironic that the studies of *E. coli* MMR have been so influential in the MMR field given that methyl-directed post-replication MMR only occurs in a small group of bacteria (see the minireview by Putnam in this issue). Below I provide a personal overview of eukaryotic MMR including my views of the history of MMR, what we know about MMR mechanisms, the role of MMR defects in cancer and questions that remain to be answered. However, I refer the reader to the work of the authors contributing articles to this volume as well as other papers for the details and the many aspects of MMR, including *E. coli* MMR, that I have not had space to cover in detail.

The nomenclature for MMR genes/proteins is confusing because the human homolog of *Saccharomyces cerevisiae PMS1* was named *PMS2* and the human homolog of *S. cerevisiae MLH2* was named *PMS1*. In this article, I have generally used *S. cerevisiae* gene/protein names. However, in places where it was important to specifically refer to a human or mouse gene/protein, I have

included the suffixes sc (*S. cerevisiae*), h (human) and mus (mouse) to help prevent confusion. A useful summary table of MMR genes, proteins and protein complexes has been published recently [17] (see the minireview by Putnam in this issue).

2. Identification of eukaryotic mismatch repair genes and proteins

Having already developed studies of HR in the yeast *S. cerevisiae*, my lab initiated studies on *S. cerevisiae* MMR because of two observations suggesting that eukaryotic MMR might be quite different from *E. coli* MMR. First, *S. cerevisiae* was found to have no DNA methylation and therefore could not have methyl-directed MMR [18, 19]. Second, with the development of the double strand break repair (DSB/DSBR) model of HR [20], it was proposed that DSBR could account for the relationship between gene conversion and post-meiotic segregation of genetic markers without the need for classical MMR [20]. Thus, it was proposed that gene conversion would occur when a genetic marker was located in the region near the DSB that was degraded during DSBR to form a double strand gap eliminating the mutant or wild-type allele, and post-meiotic segregation would occur when the genetic marker was located in the region of heteroduplex DNA flanking the DSB during DSBR, thus resulting in a mispair that was then not acted on by MMR. Further, post-replication MMR could be explained if an endonuclease were to make a DSB at the site of a mispair followed by DSBR with the sister DNA serving as a donor during repair [21];

endonucleases that could cleave at mispairs had been discovered [22]. This model was in contrast to more classical models where heteroduplex HR intermediates containing mispaired bases were formed and were then either repaired by MMR resulting in gene conversion or escaped repair resulting in post-meiotic segregation of the genetic marker [20]. A model illustrating these views of HR is presented in Figure 1.

To investigate whether *S. cerevisiae* had classical MMR, we adapted our previously developed *E. coli* heteroduplex plasmid transformation system [11, 12] to the study of MMR in *S. cerevisiae*. On transformation of *S. cerevisiae* with heteroduplex plasmids, we recovered plasmid products that were consistent with MMR having acted on these plasmids [23, 24]. We also showed that cell-free extracts of *S. cerevisiae* were able to catalyze repair of mispair-containing M13 phage-based heteroduplex substrates [25]; other eukaryotic cell-free MMR systems were subsequently developed by others [26-28]. At this time we were aware of mutations identified in Seymour Fogel's lab named *pms* mutations, for Post-Meiotic Segregation, which caused a decrease in gene conversion events and a concomitant increase in post-meiotic segregation events [29]. Interpreted in the context of classical MMR, these results were consistent with *pms* mutations causing a MMR defect. However, interpreted in the context of the DSBR model, these results were consistent with *pms* mutations causing a decrease in the size of the double strand gap and an increase in the length of the heteroduplex regions flanking the double strand gap increasing the chance that

the position of the allele being followed would fall in the heteroduplex region flanking the DSB. We obtained *pms1* mutations from the Fogel lab and used our heteroduplex plasmid transformation assays to show that *pms1* mutations caused increased recovery of plasmid products that had escaped repair, thus establishing *pms1* mutations as causing a MMR defect and defects in processing mispairs formed during meiotic recombination to gene conversion events resulting in increased post-meiotic segregation events [30]. Our subsequent studies of Seymour's *pms1* through *pms6* mutations were of great value in helping to identify and study MMR genes. As a philosophical note, I have found trying to address this type of unanswered, possibly even somewhat controversial scientific question to be the most intellectually rewarding part of being a scientist.

Seymour's lab went on to clone the *PMS1* gene and discovered that it encoded a MutL homologue, a critical protein in *E. coli* MMR [31]. This was extremely surprising to me as we thought that eukaryotes did not have methyl-directed MMR. This motived my lab to use newly developed methods of degenerate PCR to search for *S. cerevisiae* genes encoding MutS homologues. We were able to initially identify two such MutS Homologue genes, *MSH1* and *MSH2* [32, 33]. Genetic studies suggested that *MSH1* encoded a protein that functioned in the repair of mitochondrial DNA [33]; this was surprising because the prevailing view at the time, now no longer held, was that mitochondria lacked DNA repair systems. In contrast, genetic studies showed that *msh2* defects caused increased nuclear mutation rates and increased post-meiotic segregation of

genetic markers during meiosis establishing Msh2 as the first identified eukaryotic MutS related protein [33]; *MSH2* was later found to be the gene mutated in Fogel's *pms5* mutant [34]. The Crouse laboratory identified *MSH3*, which was also known in humans and mouse, but *msh3* defects were not initially found to cause phenotypes consistent with MMR defects [35]. We also identified *MSH6* by analyzing the *S. cerevisiae* genome sequence and found that it was the gene mutated in Fogel's *pms3* and *pms6* mutants, which had not yet been extensively studied [36, 37]. The *MSH4* and *MSH5* genes (see the minireview by Manhart and Alani in this issue), encoding proteins that function in meiotic recombination but not MMR, were described by others [38, 39] and will not be discussed in this article. A key clue to the Msh proteins came from studies of human cell-free extracts that catalyzed MMR performed in the Modrich and Jiricny labs, that identified the *in vitro* human *msh2* MMR defect-complementing activity as a two subunit protein complex containing Msh2 and a fragment of GTBP (also called Msh6) [40, 41]. My lab performed two-hybrid interaction studies showing that Msh2 was the common subunit of the Msh2-Msh3 and Msh2-Msh6 complexes [36]. We further showed that these complexes were partially redundant with each other, consistent with the idea based on the function of bacterial MutS that these complexes were mispair recognition proteins with partially overlapping mispair-binding specificity [36]. Other genetic studies provided additional details about the predicted mispair recognition specificity of these two complexes [42]. Collaborative biochemical studies with the Fishel lab as well as biochemical studies by others quickly extended this model to the

human MSH complexes [43, 44] and studies of mutant mice and mouse cell lines as well as human tumor cell lines provided supporting genetic evidence [45-49].

The discovery and characterization of the MutL Homologue or MLH complexes was similar to that of the MSH complexes. *S. cerevisiae MLH1* was discovered by the Liskay lab using degenerate PCR similar to the discovery of *MSH2* [50] and later found to be the gene mutated in Fogel's *pms2* and *pms4* mutants [51]. Genetic studies showed that defects in *MLH1* and *PMS1* caused very similar mutator phenotypes [50] and, consistent with this early biochemical studies, established the existence of a Mlh1-Pms1 complex that could be recruited by Msh2 [52]. Protein fractionation studies identified the human *in vitro mlh1* MMR defect-complementing activity as a two-subunit complex containing Mlh1 and Pms2 (Pms2 is the human homologue of *S. cerevisiae* Pms1) [53]. The other two *S. cerevisiae MLH* genes, *MLH2* and *MLH3* were identified through the analysis of the *S. cerevisiae* genome sequence as well as using two-hybrid screens [54-56]. Like Pms1, the Mlh3 protein forms a complex with Mlh1, and genetic studies have indicated that the Mlh1-Mlh3 complex plays a minor role in MMR by substituting for Mlh1-Pms1 in the repair of some types of mispairs [55, 56], as have biochemical studies of hMlh1-Mlh3 [57]. The Mlh1-Mlh3 complex also plays a role in meiotic recombination in conjunction with the Msh4-Msh5 complex [54, 58, 59]. The Mlh1-Mlh2 complex has remained enigmatic. Genetic studies in both *S. cerevisiae* and mouse as well as biochemical complementation studies utilizing human *in vitro* MMR reactions initially provided little evidence for

a role in MMR [56, 60, 61] although some studies have suggested Mlh2 might play a minor role in meiotic recombination [54, 59]. However, recent studies have indicated that Mlh1-Mlh2 is more important for MMR when Mlh1-Pms1 levels are reduced suggestive of a different role in MMR than the other MutL homologue complexes [62].

The other genes/proteins thought to be involved in MMR include Exonuclease 1 (Exo1), Proliferating Cell Nuclear Antigen (PCNA), Replication Factor C (RFC), Replication Factor/Protein A (RPA) and DNA polymerase δ (DNA Pol δ). Exo1 was first described as a meiotic HR 5' to 3' exonuclease in *S. pombe* [63, 64]. A role for Exo1 in MMR in *S. cerevisiae* was discovered through the isolation of *EXO1* as encoding an Msh2-interacting protein in a two-hybrid screen [65] and was subsequently found to also interact with Mlh1 [66, 67]. While Exo1 is thought to play a key role in the excision step of MMR, genetic studies have shown that Exo1 is not essential for MMR and that other excision mechanisms must exist [65, 68, 69]. PCNA was implicated in MMR through its role in the resynthesis step of MMR, through protein-protein interaction studies, and the identification of mutations in the *S. cerevisiae POL30* gene encoding PCNA, which cause MMR defects and a mutator phenotype [70-73]. Subsequently, PCNA was shown to play a role in coupling MMR to DNA replication through interactions with PIP (PCNA-interacting peptide)-box motifs located in the N-termini of Msh3 and Msh6 [74-77]. PCNA also interacts with scMlh1-Pms1 and hMlh1-Pms2 [78, 79], and this interaction could play a role in activating the

endonuclease activity of these 2 complexes [80, 81]. The PCNA loading factor RFC was implicated in MMR through the requirement for PCNA, and the identification of a MMR-defective mutation in one of the genes encoding RFC has provided genetic support for this requirement [82]. DNA Pol δ was implicated in MMR through *in vitro* reconstitution studies [83], but a possible role for other DNA polymerases has not been ruled out. Similarly, RPA was implicated in MMR through *in vitro* reconstitution studies [84-86]. Some studies have suggested that HMGB, PARP1, RFX and most recently the MCM8-MCM9 complex may play a role in MMR, but the evidence for these is in my view not yet well established [87-90]. A model summarizing the known MMR proteins is presented in Figure 2.

3. Biochemical activities of MMR proteins

The presently known MMR proteins fall into two groups. One consists of proteins that function in many aspects of DNA metabolism including DNA replication and recombination, such as Exo1, RPA, PCNA, RFC and DNA Pol δ. These proteins have been extensively characterized in a variety of contexts and will not be generally commented on here. The second consists of proteins that are more specific to MMR, including the MutS-related heterodimers Msh2-Msh6 and Msh2-Msh3 and the MutL-related heterodimers Mlh1-Pms1 (Mlh1-Pms2 in human), Mlh1-Mlh3 and Mlh1-Mlh2 (Mlh1-Pms1 in human) (see the minireview by Putnam in this issue). Many of these proteins have been extensively characterized using a diversity of techniques beyond more standard biochemical studies including X-

ray crystallography, small angle X-ray scattering, deuterium-exchange mass spectrometry, imaging proteins *in vivo* and single molecule biochemistry approaches, many of which have been coupled to different types of genetics studies [91, 92](see the minireviews by Groothuizen and Sixma, by Friedhoff and Gotthardt and by Schmidt and Hombauer in this issue). The application of these approaches has been exciting to follow and has brought many new insights to the field.

The Msh2-Msh6 and Msh2-Msh3 mispair-recognition complexes have been shown to form a ring around DNA and under certain conditions form a sliding clamp structure, a conformation that the Fishel lab has extensively studied and incorporated into specific models for MMR mechanisms [93, 94] (see the minireview by Hingorani in this issue). In the absence of nucleotide or in the presence of ADP, these mispair recognition factors appear to form an unstable ring on base paired DNA and similarly form an unstable ring at the site of the mispair in mispaired DNA [95]. On addition of ATP, the proteins bound to base paired DNA dissociate whereas the mispair-bound proteins are converted to a stable clamp that then slides along the DNA and, on linear DNAs, dissociates from the ends of the DNA [93, 96-98]. The pioneering X-ray crystallography studies of the bacterial MutS proteins performed by Sixma [99] as well as Hsieh and Yang [100], followed later by structural studies of the eukaryotic Msh complexes [101, 102] as well as structure-function genetics studies have contributed enormously to the understanding of the mispair-bound forms of these

proteins. The Msh2-Msh6 and Msh2-Msh3 complexes can also recruit Mlh1-Pms1 and Mlh1-Mlh2, and most likely Mlh1-Mlh3, in a mispair-dependent, ATP-dependent reaction corresponding to the conditions where sliding clamps form [62, 95, 97, 103-107]. However, while mutations that disrupt sliding clamp formation also disrupt MMR [37, 108, 109], it is not clear at which step of MMR sliding clamps are required because at least one sliding clamp-defective Msh2-Msh6 mutant is proficient for recruiting and activating the Mlh1-Pms1 endonuclease and promotes Mlh1-Pms1 endonuclease-dependent MMR reactions *in vitro* [110]. It is also possible that sliding clamps reflect an ATP-induced conformational change required for MMR with sliding clamps being predominantly seen in the absence of other MMR proteins. Many studies have suggested that a stable 1:1 complex of an Msh complex with an Mlh complex plays a role in MMR (for representative papers see [97, 103, 104, 106, 107]). In contrast, imaging of MMR proteins in living cells has suggested that Msh2-Msh6, and likely Msh2-Msh3, can act to catalytically load multiple molecules of Mlh1-Pms1 and Mlh1-Mlh2 (and possibly Mlh1-Mlh3) onto DNA in a mispair-dependent reaction [62, 77]. Determining if the Msh complexes can catalytically load Mlh complexes onto DNA in reconstituted biochemical reactions and then elucidating the biochemical activities of the Msh complex-free Mlh complexes will be critical to decipher the mechanism of the early steps in MMR.

The function of the eukaryotic MutL-related complexes, Mlh1-Pms1, Mlh1-Mlh3 and Mlh1-Mlh2 remained poorly understood for approximately twelve years after

the first such complex, Mlh1-Pms1, was discovered. Then the Modrich lab demonstrated that both the human hMlh1-Pms2 complex and its *S. cerevisiae* homolog scMlh1-Pms1 have an endonuclease activity [70, 111] (see the minireview by Kadyrova and Kadyrov in this issue). The endonuclease active site has been extensively characterized by structure-function genetics and X-ray crystallography [110, 112, 113]. This endonuclease has very weak activity on its own but can be activated to nick supercoiled, base paired DNA by PCNA and RFC and can be more strikingly activated by Msh2-Msh6 (or Msh2-Msh3), PCNA and RFC to nick mispaired DNA containing a pre-existing nick. Remarkably, in this latter 4-protein nicking reaction, the Mlh1-Pms1 complex is highly specific for nicking the already nicked strand [70, 80, 110, 111]. Not only is the molecular mechanism for this strand-specific nicking unknown, a role for such a mechanism in MMR seems paradoxical given that pre-existing nicks are often present in newly replicated DNA, in particular during lagging strand replication and these can potentially serve as sites for the initiation of excision reactions. It is possible that such a mechanism simply increases the probability of nicks being present 5' to mispairs to act as substrates for Exo1 or other excision processes, or it is possible that this mechanism reflects other excision mechanisms possibly mediated by high-density nicking at or near the mispair [81, 114]. Sequence homology relationships suggested that Mlh1-Mlh3 would have an endonuclease activity (see the minireview by Putnam in this issue) and this has been demonstrated, although the properties of the Mlh1-Mlh3 endonuclease thus far reported are strikingly different from those of the Mlh1-Pms1 endonuclease [115,

116]. The Mlh1-Mlh2 complex is not predicted to have an endonuclease activity (see the minireview by Putnam in this issue) and its possible role in MMR has remained unclear. Surprisingly, Mlh1-Mlh2 shares many properties with Mlh1-Pms1 including mispair-dependent recruitment by Msh2-Msh6 and Msh2-Msh3 and mispair- and Msh2-Msh6-dependent formation of foci in living cells, suggesting that Mlh1-Msh2 may play some type of accessory role in MMR [62]. Much remains to be learned about Mlh1-Mlh2 and Mlh1-Mlh3 in comparison to what we know about Mlh1-Pms1.

4. Reconstitution of MMR *in vitro*

The demonstration that extracts of *S. cerevisiae*, Xenopus, Drosophilia, human and mouse cells could catalyze repair of mispair-containing DNAs [25-28, 69] set the stage for biochemical fractionation and reconstitution studies demonstrating that eukaryotic MMR proteins can catalyze a nick-directed MMR reaction similar to the reaction catalyzed by *E. coli* MMR proteins. The most rapid progress was made in the human system, largely due to work performed in the Modrich and Li labs. However, I am pleased that my lab was ultimately able to reconstitute MMR reactions using purified *S. cerevisiae* MMR proteins, making it possible to apply the wealth of insights from genetics studies of MMR in *S. cerevisiae* to the biochemistry of reconstituted MMR reactions. To date, reconstitution studies have defined two types of mispair-directed excision/repair reactions. In one reaction, a combination of Msh2-Msh6 or Msh2-Msh3, Exo1, DNA Pol δ, the

single-stranded DNA binding protein RPA, PCNA and RFC promotes the repair of a circular mispaired substrate containing a nick on the 5' side of the mispair. In this reaction, the mispair recognition factors and other proteins promote excision by Exo1 past the mispair, followed by repair DNA synthesis to fill in the resulting gap repairing the initial mispair [85, 86, 117]. In a second reaction, a combination of Msh2-Msh6 or Msh2-Msh3, Mlh1-Pms1 (hMlh1-Pms2), Exo1, DNA Pol δ, RPA, PCNA and RFC promotes the repair of a circular mispaired substrate containing a nick on the 3' side of the mispair. In this reaction, the Mlh1-Pms1 endonuclease is activated to generate nicks 5' to the mispair, which then allows Exo1-dependent 5' excision and subsequent gap filling to occur essentially as in the 5'-nick-directed *in vitro* MMR reactions [86, 110]. In both of these reactions, mispair recognition by Msh2-Msh6 and Msh2-Msh3 appears to stimulate excision by Exo1, in part by overcoming inhibition of Exo1 by RPA [118]. Whether the ability of Exo1 to interact with Msh2, and potentially with the Msh2-Msh6 and Msh2-Msh3 sliding clamps, plays a role in this stimulation of Exo1 is unclear. Mlh1-Pms1 does not stimulate excision in reconstituted 5'-nick-directed MMR reactions even though hMlh1-Pms2 appears to be required for maximal 5'-nick-directed excision in human extract-based MMR reactions under conditions where the hMlh1-Pms2 endonuclease should not be required [119].

A critical question about the reconstituted MMR reactions discussed above is how well they correspond to MMR *in vivo*. It seems likely that these reactions recapitulate nick-directed Exo1-mediated mispair excision and coupled gap-filling

by DNA Pol δ that functions in MMR, particularly on the lagging strand, which is expected to have a high density of nicks due to discontinuous DNA synthesis. It is not clear whether other DNA polymerases besides DNA Pol δ can participate in the gap-filling reaction. In contrast, genetic studies in *S. cerevisiae* and mice have shown that Exo1 defects only cause small defects in MMR and that redundant excision mechanisms exist [65, 69, 120]. Strikingly, all known mutations that specifically inactivate Exo1-independent MMR but not Exo1-dependent MMR that have been characterized to date are highly but not completely defective for activation of the Mlh1-Pms1 endonuclease [68, 81, 110]. These mutations all cause defects in reconstituted Mlh1-Pms1-dependent MMR reactions suggesting that these reactions could represent coupling of the Exo1-independent MMR Mlh1-Pms1 activation pathway to an Exo1-dependent excision system [110]. These results also suggest that high levels of Mlh1-Pms1 endonuclease activation are required for Exo1-independent MMR, raising the possibility that high levels of Mlh1-Pms1 endonuclease activation could represent an endonuclease-promoted mispair excision pathway [81, 114]. Other possible Exo1-independent mispair excision mechanisms have also been proposed [120, 121]. Finally, MMR is coupled to DNA replication *in vivo* [77, 122], but little is known about how the MMR mechanisms defined *in vitro* are coupled to DNA replication.

5. Genetics of mammalian MMR defects

My initial interest in a possible relationship between MMR defects and human disease came from the similarity between the phenotypes caused by defects in *S. cerevisiae MSH1* and some of the human mitochondrial myopathies; this idea did not prove to be relevant as mammals do not appear to contain a gene encoding an Msh1 protein. Regardless, I set out to try to clone human and mouse *MSH2* in collaboration with Rick Fishel and later to clone human *MLH1* and *PMS1* (known in the literature as *PMS2*) by extending the collaboration to include Mike Liskay. I knew that defects in MMR genes caused microsatellite instability due to the published work on *E. coli* MMR and because I had given my *msh2* mutations to Tom Petes for his studies on microsatellite instability in *S. cerevisiae* [123, 124]. We had already cloned the human *MSH2* gene when reports appeared on microsatellite instability in human tumors and in tumors associated with Hereditary Non-polyposis Colorectal Cancer (HNPCC, now called Lynch Syndrome) [125, 126], causing considerable excitement. We rapidly mapped the *MSH2* gene to the Chromosome 2 HNPCC locus, setting off our search for evidence that *MSH2* was mutated in HNPCC [127, 128]. The chromosome 3 HNPCC locus was reported a few months later [129] leading to our subsequent studies demonstrating that *MLH1* was the chromosome 3 HNPCC gene [130, 131]. We focused on these two genes because they were the only two genes that could be linked to HNPCC by mapping studies. Similar studies were performed de la Chapelle and Vogelstein [132, 133]. It was exciting for me to learn how to clone and determine the structure of mammalian genes and to set up molecular diagnostics assays. I was particularly grateful to my colleagues at

the Dana-Farber Cancer Institute who worked very hard to help identify collaborators who could provide samples from HNPCC families for analysis. I was also grateful to be able to work with these clinical collaborators, whose efforts were essential in ultimately showing that *MSH2* and *MLH1* were the major HNPCC genes and in bringing these genes into the area of molecular diagnostics of cancer predisposition. A particularly striking moment occurred when a mutation carrier identified in our initial studies who did not have cancer was re-examined by their physician and found to have a previously undetected cancer, thereby potentially saving her life; this impressed on me the true importance of finding ways to use basic science discoveries to impact human health.

Many laboratories rapidly started working on the relationship between MMR defects and cancer susceptibility, both through studies of human patients and the establishment of mouse models (see the minireviews by Heinen, by Begum and Martin, by Peña-Diaz and Rasmussen, by Sijmons and Hofstra and by Lee, Tosti and Edelmann in this issue). My lab focused on further developing the genetics and biochemistry of *S. cerevisiae* MMR, which has provided an excellent foundation for studies of the genetics of MMR in human and mouse, the development of molecular diagnostics for defects in *MSH2* and *MLH1* as these were clearly the major Lynch Syndrome genes (they were also the only cancers we had good access to), and providing the first evidence for silencing of *MLH1* in sporadic MMR-defective cancers and for *MLH1* epimutations [128, 131, 134, 135]. Studies from other labs established that *hPMS2* and *MSH6* were also

defective in cancer, albeit in cancers/syndromes that seem somewhat different from classical Lynch syndrome, whereas defects in *hPMS1* (*scMLH2*), *MLH3* and *EXO1* were not convincingly demonstrated or were very rare, consistent with the weak mutator phenotypes caused by defects in these genes in model organisms (For representative papers see [136-140]). Studies of human cancer cell lines containing MMR defects initially characterized in the Karran and Kunkel labs as well as by others also helped establish the nature of human MMR defects and the relationships between MMR and DNA damage responses [141-145]. The first mouse models of MMR defects were produced by the Liskay (*mPMS2*) and Mak (*MSH2*) labs and de Wind working with te Riele (*MSH2*); these were rapidly followed by studies of other mouse MMR genes by Edelmann and Kucherlapati (*MLH1, MSH6, MSH3, EXO1*), Liskay (*MLH1*), de Wind and te Riele (*MSH6, MSH3*) and Lipkin (*MLH3*) [45-47, 60, 69, 146-151] (see the minireview by Lee, Tosti and Edelmann in this issue). The extensive efforts in human and mouse genetics, most recently incorporating data generated by cancer genome sequencing projects such as The Cancer Genome Atlas Project have provided a broad picture of the contribution of human MMR defects to cancer susceptibility; it is gratifying to me that studies in *S. cerevisiae* have provided such an accurate road map for the study of the mammalian MMR genes attesting to the importance of studies in model organisms. In the long run, I hope that understanding MMR defects in cancer will lead to treatments for MMR-defective cancers and in this regard, recent reports that MMR defects may predict responses to immunotherapy are cause for optimism [152, 153].

6. Unanswered or incompletely answered big-picture questions about MMR mechanisms

While we have learned a great deal about eukaryotic MMR, there is still much that we do not know. The unanswered questions about MMR appear to fall in three general areas. First, what are the detailed reaction mechanisms of individual MMR proteins and key groups of MMR proteins that functionally interact? Second, how do we reconstitute complete MMR reactions that reflect coupling to DNA replication, processing of chromatin substrates and how are strand-discrimination signals produced and recognized? Third, how do MMR proteins function in other processes beyond post-replication MMR such as preventing recombination between divergent DNA sequences and the DNA damage response?

The MMR proteins are a fascinating group of proteins and individual activities have been linked to each protein and studied in almost all cases. A striking property of virtually all known MMR proteins is that their function in MMR is driven by different conformational changes and different protein-protein interactions. Yet how these interactions then modulate the activities of each MMR protein seems less clear. Some examples include: 1) do interactions of Exo1 with Msh2-Msh6 (or Msh2-Msh3) and/or Mlh1-Pms1 play a role in recruiting and activating Exo1; 2) how does Mlh1-Pms1 interact with its activating proteins

so that its nicking activity is directed to the already nicked strand; 3) does Mlh1-Pms1 function as a stable complex with Msh2-6 (or Msh2-Msh3) or is it loaded onto the DNA and then acts independently of the mispair recognition proteins; 4) does Mlh1-Mlh2 stimulate the activity of Mlh1-Pms1 and if so, how; and 5) how does the Mlh1-Mlh3 endonuclease function in MMR given that its activity seems so different from that of Mlh1-Pms1. These types of questions as well as whether post-translational modifications modulate the activity of MMR proteins and whether there are MMR proteins yet to be identified will likely be addressed over the next few years using a combination of genetics and biophysical methods.

Tremendous progress has been made in identifying eukaryotic MMR proteins, elucidating their activities and reconstituting MMR reactions *in vitro*, although many questions about MMR mechanisms remain to be answered. These reconstituted MMR reactions in many ways mimic the mechanisms of *E. coli* MMR reactions, despite the greater number of MutS-related and MutL-related heterodimeric complexes involved in eukaryotic MMR. However, eukaryotic MMR does not use post-replication DNA methylation asymmetries as a strand specificity signal, and the eukaryotic strand specificity signal has not been definitively established. An attractive hypothesis that has existed for many years due to the fact that MMR proteins catalyze nick-directed MMR is that nicks in the newly replicated strand due to discontinuous DNA synthesis serve as a strand specificity signal [27, 154]. In addition, it has been suggested that PCNA loaded at these nicks or remaining at these nicks after DNA synthesis [155] plays a role

in strand specificity [74, 156], which is especially attractive given the activation of the nick-directed Mlh1-Pms1 (hMLH1-PMS2) endonuclease by PCNA loaded at nicks [80]. This hypothesis is most applicable to the lagging strand where nicks are expected to be prevalent; however, there is currently little direct evidence for this mechanism of strand recognition and it raises the question of what is the exact role of the Mlh1-Pms1 (hMLH1-PMS2) endonuclease, given that nicks are already present in the DNA. This hypothesis is likely less applicable to the leading strand that is synthesized more continuously and where PCNA is less important in DNA synthesis compared to its role in Pol δ-dependent lagging strand DNA synthesis [157]. Nicking of misincorporated ribonucleotides by RNase H2 has been postulated to provide a strand specificity signal that could act on the leading strand [158, 159]; however, the mutator phenotype caused by RNase H2 defects seem too weak for this to be a major strand discrimination mechanism [160]. MMR is also coupled to DNA replication, which could aid in the recognition of rarely occurring mispairs *in vivo* [77, 122](for a review see [161]). This coupling occurs by at least two different mechanisms, one of which utilizes the interaction between PCNA and the N-terminus of either Msh6 or Msh3 whereas the other mechanism(s) has not been identified [74, 75, 77]. There is some evidence that an interaction between the deuterostome-specific Msh6 PWWP domain (not present in Msh3) and a histone modification can help target Msh2-Msh6 to chromatin during S-phase, although PWWP domain mutations have not been tested to verify the significance of this interaction [162]. Finally, MMR must act on chromatin and interact with chromatin dis-assembly

and assembly pathways as well as other pathways that process replication intermediates [163-166]. It is possible that studies focused on using purified proteins to reconstitute MMR reactions that are coupled to DNA replication and chromosome dis-assembly and assembly *in vitro* could provide new insights into MMR mechanisms and how MMR is targeted to the newly synthesized DNA strands.

Much of the investigation of MMR has focused on the enzymatic mechanisms of post-replication MMR and mutagenesis mechanisms involving MMR. Yet MMR proteins function in other processes. One example is the role of MMR proteins along with other proteins such as the Sgs1 DNA helicase in preventing recombination between divergent DNA sequences, a reaction that was first described by Radman and Huang [167-170] (see the minireview by Tlam, Kanaar and Lebbnik in this issue). This process seems likely to play an important role in preventing non-allelic homologous recombination, which might otherwise lead to genome rearrangements [171]. Little is yet known about the enzymatic mechanisms of this process, although the recent demonstration that Msh2-Msh6 can recognize mispairs in the context of Rad51-mediated D-loops represents important progress [172]. Similarly, the mechanism by which MMR proteins act on mispairs in the intermediates formed during normal homologous recombination increasing gene conversion and decreasing post-meiotic segregation has not been extensively studied. MMR proteins also act in the processes that promote the expansion of trinucleotide repeats [173, 174], an

important human disease process; however, the role of MMR proteins in this process has not been fully elucidated (see the minireview by Schmidt and Pearson in this issue). Finally, MMR proteins play a role in DNA damage signaling, in promoting cell death in response to DNA damaging agents such as chemotherapeutic agents and in suppressing mutations induced by DNA damage as well as promoting mutagenesis (For representative reviews see [144, 175-177])(see the minireviews by Li, Pearlman and Hsieh, by Crouse, and by Zanotti and Gearhart in this issue). Considerable data have been accumulated on the interaction between MMR proteins and signaling proteins, and many cell biology studies have documented defects in cell death mechanisms in MMR-defective mutants. However, the MMR-dependent cell death mechanisms such as direct signaling to initiate cell death or the induction of futile cycles of DNA repair have not been fully elucidated. These important MMR-related pathways all warrant further study.

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Figure Legends

Figure 1. Double strand break repair models illustrating potential roles of mismatch repair in recombination and potential roles of double strand break repair in mismatch repair. A. DSBR model that explains how gene conversion can occur without the need to invoke MMR. One (the a allele) of the two alleles (a allele-containing chromosome in blue and A allele-containing chromosome in red) whose segregation during DSBR is illustrated is located on the recipient chromosome at the location where the DSB occurs and is then expanded into a gap with 3' single strand overhangs thus eliminating the a allele. Strand invasion to form a D-loop followed by priming of repair synthesis (dashed lines) and strand displacement, capture of the other end of the DSB and additional repair DNA synthesis result in the formation of a double Holliday junction intermediate. Resolution of the two Holliday junctions in the non-crossover directions yields two A allele-containing chromosomes and hence a gene conversion event. Other resolution directions are possible, but these do not alter the illustrated gene conversion event. **B.** An alternative of the DSBR model illustrated in A where one (the a allele) of the two alleles whose segregation during DSBR is illustrated is located on the recipient chromosome in the region that is converted to a 3' single strand overhang. This then results in the formation of a heteroduplex region containing an A:a mispair on one of product chromosomes whereas repair synthesis recreates the starting allele on both strands of the other produce chromosome. If DNA replication then occurs

without MMR (Left) Post-Meiotic Segregation results whereas if MMR occurs prior to DNA replication as illustrated (Right), then gene conversion occurs. **C.** The DSBR model can account for post-replication repair of mispairs if a mispair (illustrated as a:A) generated as the result of a replication error is simply cleaved by a mismatch-specific endonuclease to generate a DSB. Then DSBR will result in copying the wild-type allele into the broken chromosome effectively producing the same outcome as classical excision repair mechanisms of MMR.

Figure 2. Illustration summarizing the known eukaryotic proteins that function in mismatch repair. An MMR pathway involving the Msh2-Msh6 mispair recognition complex, the Mlh1-Pms1 endonuclease, Exo1, PCNA, the RFC clamp loader, RPA and DNA Pol δ is illustrated on the left side of the figure. Interactions between Msh2 and Mlh1, between Exo1 and both Msh2 and Mlh1, and between PCNA and the N-terminus of Msh6 are indicated. The Mlh1-Mlh2 complex, which is not an endonuclease, is indicated because it appears to have a role in MMR when the level of Mlh1-Pms1 is experimentally reduced. Substitution of the Msh2-Msh3 mispair recognition complex for Msh2-Msh6 is illustrated in the center and on the right, and substitution of the Mlh1-Mlh3 complex for the Mlh1-Pms1 complex in MMR reactions involving Msh2-Msh3 is illustrated to the right (note that it has not been resolved whether Mlh1-Mlh3 can also function in conjunction with Msh2-Msh6). The thickness of the arrows connecting each pathway to the three classes of mispairs illustrated reflects the relative importance of each pathway in repair of the different classes of mispairs.

The color-coding indicates whether the different genes are essential or are redundant/partially redundant with each other and hence whether loss-of-function mutations in the different genes result in complete or partial loss of MMR or inviability. All gene/protein names are *S. cerevisiae* gene/protein names.

References

[1] R.A. Holliday, A mechanism for gene conversion in fungi, Genetic research, 5 (1964) 282-304.

[2] E.M. Witkin, N.A. Sicurella, Pure Clones of Lactose-Negative Mutants Obtained in Escherichia Coli after Treatment with 5-Bromouracil, Journal of molecular biology, 8 (1964) 610-613.

[3] J. Wildenberg, M. Meselson, Mismatch repair in heteroduplex DNA, Proceedings of the National Academy of Sciences of the United States of America, 72 (1975) 2202-2206.

[4] R. Wagner, Jr., M. Meselson, Repair tracts in mismatched DNA heteroduplexes, Proceedings of the National Academy of Sciences of the United States of America, 73 (1976) 4135-4139.

[5] B. Glickman, P. van den Elsen, M. Radman, Induced mutagenesis in dam- mutants of Escherichia coli: a role for 6-methyladenine residues in mutation avoidance, Molecular and general genetics, 163 (1978) 307-312.

[6] B.W. Glickman, M. Radman, Escherichia coli mutator mutants deficient in methylation-instructed DNA mismatch correction, Proceedings of the National Academy of Sciences of the United States of America, 77 (1980) 1063-1067.

[7] B.R. McGraw, M.G. Marinus, Isolation and characterization of Dam+ revertants and suppressor mutations that modify secondary phenotypes of dam-3 strains of Escherichia coli K-12, Molecular and general genetics, 178 (1980) 309-315.

[8] E.C. Siegel, V. Bryson, Mutator gene of Escherichia coli B, Journal of bacteriology, 94 (1967) 38-47.

[9] M. Radman, R. Wagner, Mismatch repair in Escherichia coli, Annual review of genetics, 20 (1986) 523-538.

[10] P. Modrich, DNA mismatch correction, Annual review of biochemistry, 56 (1987) 435-466.

[11] R. Fishel, R. Kolodner, Gene conversion in Escherichia coli: the identification of two repair pathways for mismatched nucleotides, in: E. Friedberg, B. Bridges (Eds.) UCLA Symposia on Molecular and Cellular Biology, Alan R. Liss, Inc., New York, 1983, pp. 309-324.

[12] R.A. Fishel, R. Kolodner, An Escherichia coli cell-free system that catalyzes the repair of symmetrically methylated heteroduplex DNA, Cold Spring Harb Symp Quant Biol, 49 (1984) 603-609.

[13] R.S. Lahue, K.G. Au, P. Modrich, DNA mismatch correction in a defined system, Science, 245 (1989) 160-164.

[14] A.L. Lu, S. Clark, P. Modrich, Methyl-directed repair of DNA base-pair mismatches in vitro, Proceedings of the National Academy of Sciences of the United States of America, 80 (1983) 4639-4643.

[15] P. Modrich, Mechanisms and biological effects of mismatch repair, Annual review of genetics, 25 (1991) 229-253.

[16] P. Modrich, Methyl-directed DNA mismatch correction, The Journal of biological chemistry, 264 (1989) 6597-6600.

[17] G.X. Reyes, T.T. Schmidt, R.D. Kolodner, H. Hombauer, New insights into the mechanism of DNA mismatch repair, Chromosoma, (2015).

[18] S. Hattman, C. Kenny, L. Berger, K. Pratt, Comparative study of DNA methylation in three unicellular eucaryotes, Journal of bacteriology, 135 (1978) 1156-1157.

[19] J.H. Proffitt, J.R. Davie, D. Swinton, S. Hattman, 5-Methylcytosine is not detectable in Saccharomyces cerevisiae DNA, Molecular and cellular biology, 4 (1984) 985-988.

[20] J.W. Szostak, T.L. Orr-Weaver, R.J. Rothstein, F.W. Stahl, The double-strand-break repair model for recombination, Cell, 33 (1983) 25-35.

[21] R. Wagner, C. Dohet, M. Jones, M.P. Doutriaux, F. Hutchinson, M. Radman, Involvement of Escherichia coli mismatch repair in DNA replication and recombination, Cold Spring Harb Symp Quant Biol, 49 (1984) 611-615.

[22] W.K. Holloman, T.C. Rowe, J.R. Rusche, Studies on nuclease alpha from Ustilago maydis, The Journal of biological chemistry, 256 (1981) 5087-5094.

[23] D.K. Bishop, J. Andersen, R.D. Kolodner, Specificity of mismatch repair following transformation of Saccharomyces cerevisiae with heteroduplex plasmid DNA,

Proceedings of the National Academy of Sciences of the United States of America, 86 (1989) 3713-3717.

[24] D.K. Bishop, R.D. Kolodner, Repair of heteroduplex plasmid DNA after transformation into Saccharomyces cerevisiae, Molecular and cellular biology, 6 (1986) 3401-3409.

[25] C. Muster-Nassal, R. Kolodner, Mismatch correction catalyzed by cell-free extracts of Saccharomyces cerevisiae, Proceedings of the National Academy of Sciences of the United States of America, 83 (1986) 7618-7622.

[26] I. Varlet, M. Radman, P. Brooks, DNA mismatch repair in Xenopus egg extracts: repair efficiency and DNA repair synthesis for all single base-pair mismatches, Proceedings of the National Academy of Sciences of the United States of America, 87 (1990) 7883-7887.

[27] J. Holmes, Jr., S. Clark, P. Modrich, Strand-specific mismatch correction in nuclear extracts of human and Drosophila melanogaster cell lines, Proceedings of the National Academy of Sciences of the United States of America, 87 (1990) 5837-5841.

[28] D.C. Thomas, J.D. Roberts, T.A. Kunkel, Heteroduplex repair in extracts of human HeLa cells, The Journal of biological chemistry, 266 (1991) 3744-3751.

[29] M.S. Williamson, J.C. Game, S. Fogel, Meiotic gene conversion mutants in Saccharomyces cerevisiae. I. Isolation and characterization of pms1-1 and pms1-2, Genetics, 110 (1985) 609-646.

[30] D.K. Bishop, M.S. Williamson, S. Fogel, R.D. Kolodner, The role of heteroduplex correction in gene conversion in Saccharomyces cerevisiae, Nature, 328 (1987) 362-364.

[31] W. Kramer, B. Kramer, M.S. Williamson, S. Fogel, Cloning and nucleotide sequence of DNA mismatch repair gene PMS1 from Saccharomyces cerevisiae: homology of PMS1 to procaryotic MutL and HexB, Journal of bacteriology, 171 (1989) 5339-5346.

[32] R.A. Reenan, R.D. Kolodner, Isolation and characterization of two Saccharomyces cerevisiae genes encoding homologs of the bacterial HexA and MutS mismatch repair proteins, Genetics, 132 (1992) 963-973.

[33] R.A. Reenan, R.D. Kolodner, Characterization of insertion mutations in the Saccharomyces cerevisiae MSH1 and MSH2 genes: evidence for separate mitochondrial and nuclear functions, Genetics, 132 (1992) 975-985.

[34] A. Jeyaprakash, J.W. Welch, S. Fogel, Mutagenesis of yeast MW104-1B strain has identified the uncharacterized PMS6 DNA mismatch repair gene locus and

additional alleles of existing PMS1, PMS2 and MSH2 genes, Mutation research, 325 (1994) 21-29.

[35] L. New, K. Liu, G.F. Crouse, The yeast gene MSH3 defines a new class of eukaryotic MutS homologues, Molecular and general genetics, 239 (1993) 97-108.

[36] G.T. Marsischky, N. Filosi, M.F. Kane, R. Kolodner, Redundancy of Saccharomyces cerevisiae MSH3 and MSH6 in MSH2-dependent mismatch repair, Genes & development, 10 (1996) 407-420.

[37] R. Das Gupta, R.D. Kolodner, Novel dominant mutations in Saccharomyces cerevisiae MSH6, Nature genetics, 24 (2000) 53-56.

[38] P. Ross-Macdonald, G.S. Roeder, Mutation of a meiosis-specific MutS homolog decreases crossing over but not mismatch correction, Cell, 79 (1994) 1069-1080.

[39] N.M. Hollingsworth, L. Ponte, C. Halsey, MSH5, a novel MutS homolog, facilitates meiotic reciprocal recombination between homologs in Saccharomyces cerevisiae but not mismatch repair, Genes & development, 9 (1995) 1728-1739.

[40] J.T. Drummond, G.M. Li, M.J. Longley, P. Modrich, Isolation of an hMSH2-p160 heterodimer that restores DNA mismatch repair to tumor cells, Science, 268 (1995) 1909-1912.

[41] F. Palombo, P. Gallinari, I. Iaccarino, T. Lettieri, M. Hughes, A. D'Arrigo, O. Truong, J.J. Hsuan, J. Jiricny, GTBP, a 160-kilodalton protein essential for mismatch-binding activity in human cells, Science, 268 (1995) 1912-1914.

[42] E.A. Sia, R.J. Kokoska, M. Dominska, P. Greenwell, T.D. Petes, Microsatellite instability in yeast: dependence on repeat unit size and DNA mismatch repair genes, Molecular and cellular biology, 17 (1997) 2851-2858.

[43] S. Acharya, T. Wilson, S. Gradia, M.F. Kane, S. Guerrette, G.T. Marsischky, R. Kolodner, R. Fishel, hMSH2 forms specific mispair-binding complexes with hMSH3 and hMSH6, Proceedings of the National Academy of Sciences of the United States of America, 93 (1996) 13629-13634.

[44] F. Palombo, I. Iaccarino, E. Nakajima, M. Ikejima, T. Shimada, J. Jiricny, hMutSbeta, a heterodimer of hMSH2 and hMSH3, binds to insertion/deletion loops in DNA, Current biology : CB, 6 (1996) 1181-1184.

[45] W. Edelmann, A. Umar, K. Yang, J. Heyer, M. Kucherlapati, M. Lia, B. Kneitz, E. Avdievich, K. Fan, E. Wong, G. Crouse, T. Kunkel, M. Lipkin, R.D. Kolodner, R. Kucherlapati, The DNA mismatch repair genes Msh3 and Msh6 cooperate in intestinal tumor suppression, Cancer research, 60 (2000) 803-807.

[46] W. Edelmann, K. Yang, A. Umar, J. Heyer, K. Lau, K. Fan, W. Liedtke, P.E. Cohen, M.F. Kane, J.R. Lipford, N. Yu, G.F. Crouse, J.W. Pollard, T. Kunkel, M. Lipkin, R. Kolodner, R. Kucherlapati, Mutation in the mismatch repair gene Msh6 causes cancer susceptibility, Cell, 91 (1997) 467-477.

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

[48] N. Papadopoulos, N.C. Nicolaides, B. Liu, R. Parsons, C. Lengauer, F. Palombo, A. D'Arrigo, S. Markowitz, J.K. Willson, K.W. Kinzler, et al., Mutations of GTBP in genetically unstable cells, Science, 268 (1995) 1915-1917.

[49] A. Umar, J.I. Risinger, W.E. Glaab, K.R. Tindall, J.C. Barrett, T.A. Kunkel, Functional overlap in mismatch repair by human MSH3 and MSH6, Genetics, 148 (1998) 1637-1646.

[50] T.A. Prolla, D.M. Christie, R.M. Liskay, Dual requirement in yeast DNA mismatch repair for MLH1 and PMS1, two homologs of the bacterial mutL gene, Molecular and cellular biology, 14 (1994) 407-415.

[51] A. Jeyaprakash, R. Das Gupta, R. Kolodner, Saccharomyces cerevisiae pms2 mutations are alleles of MLH1, and pms2-2 corresponds to a hereditary nonpolyposis colorectal carcinoma-causing missense mutation, Molecular and cellular biology, 16 (1996) 3008-3011.

[52] T.A. Prolla, Q. Pang, E. Alani, R.D. Kolodner, R.M. Liskay, MLH1, PMS1, and MSH2 interactions during the initiation of DNA mismatch repair in yeast, Science, 265 (1994) 1091-1093.

[53] G.M. Li, P. Modrich, Restoration of mismatch repair to nuclear extracts of H6 colorectal tumor cells by a heterodimer of human MutL homologs, Proceedings of the National Academy of Sciences of the United States of America, 92 (1995) 1950-1954.

[54] T.F. Wang, N. Kleckner, N. Hunter, Functional specificity of MutL homologs in yeast: evidence for three Mlh1-based heterocomplexes with distinct roles during meiosis in recombination and mismatch correction, Proceedings of the National Academy of Sciences of the United States of America, 96 (1999) 13914-13919.

[55] H. Flores-Rozas, R.D. Kolodner, The Saccharomyces cerevisiae MLH3 gene functions in MSH3-dependent suppression of frameshift mutations, Proceedings of the National Academy of Sciences of the United States of America, 95 (1998) 12404-12409.

[56] B.D. Harfe, B.K. Minesinger, S. Jinks-Robertson, Discrete in vivo roles for the MutL homologs Mlh2p and Mlh3p in the removal of frameshift intermediates in budding yeast, Current biology : CB, 10 (2000) 145-148.

[57] E. Cannavo, G. Marra, J. Sabates-Bellver, M. Menigatti, S.M. Lipkin, F. Fischer, P. Cejka, J. Jiricny, Expression of the MutL homologue hMLH3 in human cells and its role in DNA mismatch repair, Cancer research, 65 (2005) 10759-10766.

[58] K.T. Nishant, A.J. Plys, E. Alani, A mutation in the putative MLH3 endonuclease domain confers a defect in both mismatch repair and meiosis in Saccharomyces cerevisiae, Genetics, 179 (2008) 747-755.

[59] M.F. Abdullah, E.R. Hoffmann, V.E. Cotton, R.H. Borts, A role for the MutL homologue MLH2 in controlling heteroduplex formation and in regulating between two different crossover pathways in budding yeast, Cytogenetics and genome research, 107 (2004) 180-190.

[60] T.A. Prolla, S.M. Baker, A.C. Harris, J.L. Tsao, X. Yao, C.E. Bronner, B. Zheng, M. Gordon, J. Reneker, N. Arnheim, D. Shibata, A. Bradley, R.M. Liskay, Tumour susceptibility and spontaneous mutation in mice deficient in Mlh1, Pms1 and Pms2 DNA mismatch repair, Nature genetics, 18 (1998) 276-279.

[61] M. Raschle, G. Marra, M. Nystrom-Lahti, P. Schar, J. Jiricny, Identification of hMutLbeta, a heterodimer of hMLH1 and hPMS1, The Journal of biological chemistry, 274 (1999) 32368-32375.

[62] C.S. Campbell, H. Hombauer, A. Srivatsan, N. Bowen, K. Gries, A. Desai, C.D. Putnam, R.D. Kolodner, Mlh2 is an accessory factor for DNA mismatch repair in Saccharomyces cerevisiae, PLoS genetics, 10 (2014) e1004327.

[63] P. Szankasi, G.R. Smith, A DNA exonuclease induced during meiosis of Schizosaccharomyces pombe, The Journal of biological chemistry, 267 (1992) 3014-3023.

[64] P. Szankasi, G.R. Smith, A role for exonuclease I from S. pombe in mutation avoidance and mismatch correction, Science, 267 (1995) 1166-1169.

[65] D.X. Tishkoff, A.L. Boerger, P. Bertrand, N. Filosi, G.M. Gaida, M.F. Kane, R.D. Kolodner, Identification and characterization of Saccharomyces cerevisiae EXO1, a gene encoding an exonuclease that interacts with MSH2, Proceedings of the National Academy of Sciences of the United States of America, 94 (1997) 7487-7492.

[66] P.T. Tran, J.A. Simon, R.M. Liskay, Interactions of Exo1p with components of MutLalpha in Saccharomyces cerevisiae, Proceedings of the National Academy of Sciences of the United States of America, 98 (2001) 9760-9765.

[67] C. Schmutte, M.M. Sadoff, K.S. Shim, S. Acharya, R. Fishel, The interaction of DNA mismatch repair proteins with human exonuclease I, The Journal of biological chemistry, 276 (2001) 33011-33018.

[68] N.S. Amin, M.N. Nguyen, S. Oh, R.D. Kolodner, exo1-Dependent mutator mutations: model system for studying functional interactions in mismatch repair, Molecular and cellular biology, 21 (2001) 5142-5155.

[69] K. Wei, A.B. Clark, E. Wong, M.F. Kane, D.J. Mazur, T. Parris, N.K. Kolas, R. Russell, H. Hou, Jr., B. Kneitz, G. Yang, T.A. Kunkel, R.D. Kolodner, P.E. Cohen, W. Edelmann, Inactivation of Exonuclease 1 in mice results in DNA mismatch repair defects, increased cancer susceptibility, and male and female sterility, Genes & development, 17 (2003) 603-614.

[70] F.A. Kadyrov, L. Dzantiev, N. Constantin, P. Modrich, Endonucleolytic function of MutLalpha in human mismatch repair, Cell, 126 (2006) 297-308.

[71] P.J. Lau, H. Flores-Rozas, R.D. Kolodner, Isolation and characterization of new proliferating cell nuclear antigen (POL30) mutator mutants that are defective in DNA mismatch repair, Molecular and cellular biology, 22 (2002) 6669-6680.

[72] A. Umar, A.B. Buermeyer, J.A. Simon, D.C. Thomas, A.B. Clark, R.M. Liskay, T.A. Kunkel, Requirement for PCNA in DNA mismatch repair at a step preceding DNA resynthesis, Cell, 87 (1996) 65-73.

[73] L. Gu, Y. Hong, S. McCulloch, H. Watanabe, G.M. Li, ATP-dependent interaction of human mismatch repair proteins and dual role of PCNA in mismatch repair, Nucleic acids research, 26 (1998) 1173-1178.

[74] H. Flores-Rozas, D. Clark, R.D. Kolodner, Proliferating cell nuclear antigen and Msh2p-Msh6p interact to form an active mispair recognition complex, Nature genetics, 26 (2000) 375-378.

[75] A.B. Clark, F. Valle, K. Drotschmann, R.K. Gary, T.A. Kunkel, Functional interaction of proliferating cell nuclear antigen with MSH2-MSH6 and MSH2-MSH3 complexes, The Journal of biological chemistry, 275 (2000) 36498-36501.

[76] H.E. Kleczkowska, G. Marra, T. Lettieri, J. Jiricny, hMSH3 and hMSH6 interact with PCNA and colocalize with it to replication foci, Genes & development, 15 (2001) 724-736.

[77] H. Hombauer, C.S. Campbell, C.E. Smith, A. Desai, R.D. Kolodner, Visualization of eukaryotic DNA mismatch repair reveals distinct recognition and repair intermediates, Cell, 147 (2011) 1040-1053.

[78] S.D. Lee, E. Alani, Analysis of interactions between mismatch repair initiation factors and the replication processivity factor PCNA, Journal of molecular biology, 355 (2006) 175-184.

[79] L. Dzantiev, N. Constantin, J. Genschel, R.R. Iyer, P.M. Burgers, P. Modrich, A defined human system that supports bidirectional mismatch-provoked excision, Molecular cell, 15 (2004) 31-41.

[80] A. Pluciennik, L. Dzantiev, R.R. Iyer, N. Constantin, F.A. Kadyrov, P. Modrich, PCNA function in the activation and strand direction of MutLalpha endonuclease in mismatch repair, Proceedings of the National Academy of Sciences of the United States of America, 107 (2010) 16066-16071.

[81] E.M. Goellner, C.E. Smith, C.S. Campbell, H. Hombauer, A. Desai, C.D. Putnam, R.D. Kolodner, PCNA and Msh2-Msh6 activate an Mlh1-Pms1 endonuclease pathway required for Exo1-independent mismatch repair, Molecular cell, 55 (2014) 291-304.

[82] Y. Xie, C. Counter, E. Alani, Characterization of the repeat-tract instability and mutator phenotypes conferred by a Tn3 insertion in RFC1, the large subunit of the yeast clamp loader, Genetics, 151 (1999) 499-509.

[83] M.J. Longley, A.J. Pierce, P. Modrich, DNA polymerase delta is required for human mismatch repair in vitro, The Journal of biological chemistry, 272 (1997) 10917-10921.

[84] Y.L. Lin, M.K. Shivji, C. Chen, R. Kolodner, R.D. Wood, A. Dutta, The evolutionarily conserved zinc finger motif in the largest subunit of human replication protein A is required for DNA replication and mismatch repair but not for nucleotide excision repair, The Journal of biological chemistry, 273 (1998) 1453-1461.

[85] Y. Zhang, F. Yuan, S.R. Presnell, K. Tian, Y. Gao, A.E. Tomkinson, L. Gu, G.M. Li, Reconstitution of 5'-directed human mismatch repair in a purified system, Cell, 122 (2005) 693-705.

[86] N. Constantin, L. Dzantiev, F.A. Kadyrov, P. Modrich, Human mismatch repair: reconstitution of a nick-directed bidirectional reaction, The Journal of biological chemistry, 280 (2005) 39752-39761.

[87] F. Yuan, L. Gu, S. Guo, C. Wang, G.M. Li, Evidence for involvement of HMGB1 protein in human DNA mismatch repair, The Journal of biological chemistry, 279 (2004) 20935-20940.

[88] Y. Liu, F.A. Kadyrov, P. Modrich, PARP-1 enhances the mismatch-dependence of 5'-directed excision in human mismatch repair in vitro, DNA repair, 10 (2011) 1145-1153.

[89] S. Traver, P. Coulombe, I. Peiffer, J.R. Hutchins, M. Kitzmann, D. Latreille, M. Mechali, MCM9 Is Required for Mammalian DNA Mismatch Repair, Molecular cell, 59 (2015) 831-839.

[90] Y. Zhang, F. Yuan, D. Wang, L. Gu, G.M. Li, Identification of regulatory factor X as a novel mismatch repair stimulatory factor, The Journal of biological chemistry, 283 (2008) 12730-12735.

[91] D.A. Erie, K.R. Weninger, Single molecule studies of DNA mismatch repair, DNA repair, 20 (2014) 71-81.

[92] J.B. Lee, W.K. Cho, J. Park, Y. Jeon, D. Kim, S.H. Lee, R. Fishel, Single-molecule views of MutS on mismatched DNA, DNA repair, 20 (2014) 82-93.

[93] S. Gradia, D. Subramanian, T. Wilson, S. Acharya, A. Makhov, J. Griffith, R. Fishel, hMSH2-hMSH6 forms a hydrolysis-independent sliding clamp on mismatched DNA, Molecular cell, 3 (1999) 255-261.

[94] R. Fishel, Mismatch repair, molecular switches, and signal transduction, Genes & development, 12 (1998) 2096-2101.

[95] M.L. Mendillo, V.V. Hargreaves, J.W. Jamison, A.O. Mo, S. Li, C.D. Putnam, V.L. Woods, Jr., R.D. Kolodner, A conserved MutS homolog connector domain interface interacts with MutL homologs, Proceedings of the National Academy of Sciences of the United States of America, 106 (2009) 22223-22228.

[96] D.J. Mazur, M.L. Mendillo, R.D. Kolodner, Inhibition of Msh6 ATPase activity by mispaired DNA induces a Msh2(ATP)-Msh6(ATP) state capable of hydrolysis-independent movement along DNA, Molecular cell, 22 (2006) 39-49.

[97] M.L. Mendillo, D.J. Mazur, R.D. Kolodner, Analysis of the interaction between the Saccharomyces cerevisiae MSH2-MSH6 and MLH1-PMS1 complexes with DNA using a reversible DNA end-blocking system, The Journal of biological chemistry, 280 (2005) 22245-22257.

[98] T. Wilson, S. Guerrette, R. Fishel, Dissociation of mismatch recognition and ATPase activity by hMSH2-hMSH3, The Journal of biological chemistry, 274 (1999) 21659-21664.

[99] M.H. Lamers, A. Perrakis, J.H. Enzlin, H.H. Winterwerp, N. de Wind, T.K. Sixma, The crystal structure of DNA mismatch repair protein MutS binding to a G x T mismatch, Nature, 407 (2000) 711-717.

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

[101] J.J. Warren, T.J. Pohlhaus, A. Changela, R.R. Iyer, P.L. Modrich, L.S. Beese, Structure of the human MutSalpha DNA lesion recognition complex, Molecular cell, 26 (2007) 579-592.

[102] S. Gupta, M. Gellert, W. Yang, Mechanism of mismatch recognition revealed by human MutSbeta bound to unpaired DNA loops, Nature structural & molecular biology, 19 (2012) 72-78.

[103] A. Srivatsan, N. Bowen, R.D. Kolodner, Mispair-specific recruitment of the Mlh1-Pms1 complex identifies repair substrates of the Saccharomyces cerevisiae Msh2-Msh3 complex, The Journal of biological chemistry, 289 (2014) 9352-9364.

[104] L.J. Blackwell, S. Wang, P. Modrich, DNA chain length dependence of formation and dynamics of hMutSalpha.hMutLalpha.heteroduplex complexes, The Journal of biological chemistry, 276 (2001) 33233-33240.

[105] F.S. Groothuizen, I. Winkler, M. Cristovao, A. Fish, H.H. Winterwerp, A. Reumer, A.D. Marx, N. Hermans, R.A. Nicholls, G.N. Murshudov, J.H. Lebbink, P. Friedhoff, T.K. Sixma, MutS/MutL crystal structure reveals that the MutS sliding clamp loads MutL onto DNA, Elife, 4 (2015) e06744.

[106] Y. Habraken, P. Sung, L. Prakash, S. Prakash, ATP-dependent assembly of a ternary complex consisting of a DNA mismatch and the yeast MSH2-MSH6 and MLH1-PMS1 protein complexes, The Journal of biological chemistry, 273 (1998) 9837-9841.

[107] M. Raschle, P. Dufner, G. Marra, J. Jiricny, Mutations within the hMLH1 and hPMS2 subunits of the human MutLalpha mismatch repair factor affect its ATPase activity, but not its ability to interact with hMutSalpha, The Journal of biological chemistry, 277 (2002) 21810-21820.

[108] M.T. Hess, M.L. Mendillo, D.J. Mazur, R.D. Kolodner, Biochemical basis for dominant mutations in the Saccharomyces cerevisiae MSH6 gene, Proceedings of the National Academy of Sciences of the United States of America, 103 (2006) 558-563.

[109] V.V. Hargreaves, S.S. Shell, D.J. Mazur, M.T. Hess, R.D. Kolodner, Interaction between the Msh2 and Msh6 nucleotide-binding sites in the Saccharomyces cerevisiae Msh2-Msh6 complex, The Journal of biological chemistry, 285 (2010) 9301-9310.

[110] C.E. Smith, N. Bowen, W.J.t. Graham, E.M. Goellner, A. Srivatsan, R.D. Kolodner, Activation of Saccharomyces cerevisiae Mlh1-Pms1 Endonuclease in a Reconstituted Mismatch Repair System, The Journal of biological chemistry, 290 (2015) 21580-21590.

[111] F.A. Kadyrov, S.F. Holmes, M.E. Arana, O.A. Lukianova, M. O'Donnell, T.A. Kunkel, P. Modrich, Saccharomyces cerevisiae MutLalpha is a mismatch repair endonuclease, The Journal of biological chemistry, 282 (2007) 37181-37190.

[112] E. Gueneau, C. Dherin, P. Legrand, C. Tellier-Lebegue, B. Gilquin, P. Bonnesoeur, F. Londino, C. Quemener, M.H. Le Du, J.A. Marquez, M. Moutiez, M. Gondry, S. Boiteux, J.B. Charbonnier, Structure of the MutLalpha C-terminal domain reveals how Mlh1 contributes to Pms1 endonuclease site, Nature structural & molecular biology, 20 (2013) 461-468.

[113] C.E. Smith, M.L. Mendillo, N. Bowen, H. Hombauer, C.S. Campbell, A. Desai, C.D. Putnam, R.D. Kolodner, Dominant Mutations in S. cerevisiae PMS1 Identify the Mlh1-Pms1 Endonuclease Active Site and an Exonuclease 1-Independent Mismatch Repair Pathway, PLoS genetics, 9 (2013) e1003869.

[114] E.M. Goellner, C.D. Putnam, R.D. Kolodner, Exonuclease 1-dependent and independent mismatch repair, DNA repair, 32 (2015) 24-32.

[115] L. Ranjha, R. Anand, P. Cejka, The Saccharomyces cerevisiae Mlh1-Mlh3 heterodimer is an endonuclease that preferentially binds to Holliday junctions, The Journal of biological chemistry, 289 (2014) 5674-5686.

[116] M.V. Rogacheva, C.M. Manhart, C. Chen, A. Guarne, J. Surtees, E. Alani, Mlh1-Mlh3, a meiotic crossover and DNA mismatch repair factor, is a Msh2-Msh3-stimulated endonuclease, The Journal of biological chemistry, 289 (2014) 5664-5673.

[117] N. Bowen, C.E. Smith, A. Srivatsan, S. Willcox, J.D. Griffith, R.D. Kolodner, Reconstitution of long and short patch mismatch repair reactions using Saccharomyces cerevisiae proteins, Proceedings of the National Academy of Sciences of the United States of America, 110 (2013) 18472-18477.

[118] J. Genschel, P. Modrich, Mechanism of 5'-directed excision in human mismatch repair, Molecular cell, 12 (2003) 1077-1086.

[119] H. Geng, C. Du, S. Chen, V. Salerno, C. Manfredi, P. Hsieh, In vitro studies of DNA mismatch repair proteins, Analytical biochemistry, 413 (2011) 179-184.

[120] H.T. Tran, D.A. Gordenin, M.A. Resnick, The 3'-->5' exonucleases of DNA polymerases delta and epsilon and the 5'-->3' exonuclease Exo1 have major roles in postreplication mutation avoidance in Saccharomyces cerevisiae, Molecular and cellular biology, 19 (1999) 2000-2007.

[121] F.A. Kadyrov, J. Genschel, Y. Fang, E. Penland, W. Edelmann, P. Modrich, A possible mechanism for exonuclease 1-independent eukaryotic mismatch repair, Proceedings of the National Academy of Sciences of the United States of America, 106 (2009) 8495-8500.

[122] H. Hombauer, A. Srivatsan, C.D. Putnam, R.D. Kolodner, Mismatch repair, but not heteroduplex rejection, is temporally coupled to DNA replication, Science, 334 (2011) 1713-1716.

[123] G. Levinson, G.A. Gutman, High frequencies of short frameshifts in poly-CA/TG tandem repeats borne by bacteriophage M13 in Escherichia coli K-12, Nucleic acids research, 15 (1987) 5323-5338.

[124] M. Strand, T.A. Prolla, R.M. Liskay, T.D. Petes, Destabilization of tracts of simple repetitive DNA in yeast by mutations affecting DNA mismatch repair, Nature, 365 (1993) 274-276.

[125] L.A. Aaltonen, P. Peltomaki, F.S. Leach, P. Sistonen, L. Pylkkanen, J.P. Mecklin, H. Jarvinen, S.M. Powell, J. Jen, S.R. Hamilton, et al., Clues to the pathogenesis of familial colorectal cancer, Science, 260 (1993) 812-816.

[126] P. Peltomaki, L.A. Aaltonen, P. Sistonen, L. Pylkkanen, J.P. Mecklin, H. Jarvinen, J.S. Green, J.R. Jass, J.L. Weber, F.S. Leach, et al., Genetic mapping of a locus predisposing to human colorectal cancer, Science, 260 (1993) 810-812.

[127] R. Fishel, M.K. Lescoe, M.R. Rao, N.G. Copeland, N.A. Jenkins, J. Garber, M. Kane, R. Kolodner, The human mutator gene homolog MSH2 and its association with hereditary nonpolyposis colon cancer, Cell, 75 (1993) 1027-1038.

[128] R.D. Kolodner, N.R. Hall, J. Lipford, M.F. Kane, M.R. Rao, P. Morrison, L. Wirth, P.J. Finan, J. Burn, P. Chapman, Structure of the human MSH2 locus and analysis of two Muir-Torre kindreds for msh2 mutations, Genomics, 24 (1994) 516-526.

[129] A. Lindblom, P. Tannergard, B. Werelius, M. Nordenskjold, Genetic mapping of a second locus predisposing to hereditary non-polyposis colon cancer, Nature genetics, 5 (1993) 279-282.

[130] C.E. Bronner, S.M. Baker, P.T. Morrison, G. Warren, L.G. Smith, M.K. Lescoe, M. Kane, C. Earabino, J. Lipford, A. Lindblom, et al., Mutation in the DNA mismatch repair gene homologue hMLH1 is associated with hereditary non-polyposis colon cancer, Nature, 368 (1994) 258-261.

[131] R.D. Kolodner, N.R. Hall, J. Lipford, M.F. Kane, P.T. Morrison, P.J. Finan, J. Burn, P. Chapman, C. Earabino, E. Merchant, et al., Structure of the human MLH1 locus and analysis of a large hereditary nonpolyposis colorectal carcinoma kindred for mlh1 mutations, Cancer research, 55 (1995) 242-248.

[132] F.S. Leach, N.C. Nicolaides, N. Papadopoulos, B. Liu, J. Jen, R. Parsons, P. Peltomaki, P. Sistonen, L.A. Aaltonen, M. Nystrom-Lahti, et al., Mutations of a mutS homolog in hereditary nonpolyposis colorectal cancer, Cell, 75 (1993) 1215-1225.

[133] N. Papadopoulos, N.C. Nicolaides, Y.F. Wei, S.M. Ruben, K.C. Carter, C.A. Rosen, W.A. Haseltine, R.D. Fleischmann, C.M. Fraser, M.D. Adams, et al., Mutation of a mutL homolog in hereditary colon cancer, Science, 263 (1994) 1625-1629.

[134] M.F. Kane, M. Loda, G.M. Gaida, J. Lipman, R. Mishra, H. Goldman, J.M. Jessup, R. Kolodner, Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines, Cancer research, 57 (1997) 808-811.

[135] I. Gazzoli, M. Loda, J. Garber, S. Syngal, R.D. Kolodner, A hereditary nonpolyposis colorectal carcinoma case associated with hypermethylation of the MLH1 gene in normal tissue and loss of heterozygosity of the unmethylated allele in the resulting microsatellite instability-high tumor, Cancer research, 62 (2002) 3925-3928.

[136] P. Peltomaki, Role of DNA mismatch repair defects in the pathogenesis of human cancer, Journal of clinical oncology, 21 (2003) 1174-1179.

[137] A. de la Chapelle, Genetic predisposition to colorectal cancer, Nature reviews. Cancer, 4 (2004) 769-780.

[138] K. Lagerstedt Robinson, T. Liu, J. Vandrovcova, B. Halvarsson, M. Clendenning, T. Frebourg, N. Papadopoulos, K.W. Kinzler, B. Vogelstein, P. Peltomaki, R.D. Kolodner, M. Nilbert, A. Lindblom, Lynch syndrome (hereditary nonpolyposis colorectal cancer) diagnostics, Journal of the national cancer institute, 99 (2007) 291-299.

[139] H. Hampel, W.L. Frankel, E. Martin, M. Arnold, K. Khanduja, P. Kuebler, H. Nakagawa, K. Sotamaa, T.W. Prior, J. Westman, J. Panescu, D. Fix, J. Lockman, I. Comeras, A. de la Chapelle, Screening for the Lynch syndrome (hereditary nonpolyposis colorectal cancer), New england journal of medicine, 352 (2005) 1851-1860.

[140] Y.M. Hendriks, S. Jagmohan-Changur, H.M. van der Klift, H. Morreau, M. van Puijenbroek, C. Tops, T. van Os, A. Wagner, M.G. Ausems, E. Gomez, M.H. Breuning, A.H. Brocker-Vriends, H.F. Vasen, J.T. Wijnen, Heterozygous mutations in PMS2 cause hereditary nonpolyposis colorectal carcinoma (Lynch syndrome), Gastroenterology, 130 (2006) 312-322.

[141] A. Umar, J.C. Boyer, D.C. Thomas, D.C. Nguyen, J.I. Risinger, J. Boyd, Y. Ionov, M. Perucho, T.A. Kunkel, Defective mismatch repair in extracts of colorectal and endometrial cancer cell lines exhibiting microsatellite instability, The Journal of biological chemistry, 269 (1994) 14367-14370.

[142] J.I. Risinger, A. Umar, J.C. Barrett, T.A. Kunkel, A hPMS2 mutant cell line is defective in strand-specific mismatch repair, The Journal of biological chemistry, 270 (1995) 18183-18186.

[143] P. Branch, G. Aquilina, M. Bignami, P. Karran, Defective mismatch binding and a mutator phenotype in cells tolerant to DNA damage, Nature, 362 (1993) 652-654.

[144] P. Karran, M. Bignami, DNA damage tolerance, mismatch repair and genome instability, Bioessays, 16 (1994) 833-839.

[145] A. Kat, W.G. Thilly, W.H. Fang, M.J. Longley, G.M. Li, P. Modrich, An alkylation-tolerant, mutator human cell line is deficient in strand-specific mismatch repair, Proceedings of the National Academy of Sciences of the United States of America, 90 (1993) 6424-6428.

[146] S.M. Lipkin, P.B. Moens, V. Wang, M. Lenzi, D. Shanmugarajah, A. Gilgeous, J. Thomas, J. Cheng, J.W. Touchman, E.D. Green, P. Schwartzberg, F.S. Collins, P.E. Cohen, Meiotic arrest and aneuploidy in MLH3-deficient mice, Nature genetics, 31 (2002) 385-390.

[147] S.M. Baker, C.E. Bronner, L. Zhang, A.W. Plug, M. Robatzek, G. Warren, E.A. Elliott, J. Yu, T. Ashley, N. Arnheim, R.A. Flavell, R.M. Liskay, Male mice defective in the DNA mismatch repair gene PMS2 exhibit abnormal chromosome synapsis in meiosis, Cell, 82 (1995) 309-319.

[148] A.H. Reitmair, R. Schmits, A. Ewel, B. Bapat, M. Redston, A. Mitri, P. Waterhouse, H.W. Mittrucker, A. Wakeham, B. Liu, et al., MSH2 deficient mice are viable and susceptible to lymphoid tumours, Nature genetics, 11 (1995) 64-70.

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

[150] S.M. Baker, A.W. Plug, T.A. Prolla, C.E. Bronner, A.C. Harris, X. Yao, D.M. Christie, C. Monell, N. Arnheim, A. Bradley, T. Ashley, R.M. Liskay, Involvement of mouse Mlh1 in DNA mismatch repair and meiotic crossing over, Nature genetics, 13 (1996) 336-342.

[151] W. Edelmann, P.E. Cohen, M. Kane, K. Lau, B. Morrow, S. Bennett, A. Umar, T. Kunkel, G. Cattoretti, R. Chaganti, J.W. Pollard, R.D. Kolodner, R. Kucherlapati, Meiotic pachytene arrest in MLH1-deficient mice, Cell, 85 (1996) 1125-1134.

[152] D.T. Le, J.N. Uram, H. Wang, B.R. Bartlett, H. Kemberling, A.D. Eyring, A.D. Skora, B.S. Luber, N.S. Azad, D. Laheru, B. Biedrzycki, R.C. Donehower, A. Zaheer, G.A. Fisher, T.S. Crocenzi, J.J. Lee, S.M. Duffy, R.M. Goldberg, A. de la Chapelle, M. Koshiji, F. Bhaijee, T. Huebner, R.H. Hruban, L.D. Wood, N. Cuka, D.M. Pardoll, N. Papadopoulos, K.W. Kinzler, S. Zhou, T.C. Cornish, J.M. Taube, R.A. Anders, J.R. Eshleman, B. Vogelstein, L.A. Diaz, Jr., PD-1 Blockade in Tumors with Mismatch-Repair Deficiency, New england journal of medicine, 372 (2015) 2509-2520.

[153] S. Kelderman, T.N. Schumacher, P. Kvistborg, Mismatch Repair-Deficient Cancers Are Targets for Anti-PD-1 Therapy, Cancer Cell, 28 (2015) 11-13.

[154] J.P. Claverys, S.A. Lacks, Heteroduplex deoxyribonucleic acid base mismatch repair in bacteria, Microbiological reviews, 50 (1986) 133-165.

[155] P.T. Stukenberg, J. Turner, M. O'Donnell, An explanation for lagging strand replication: polymerase hopping among DNA sliding clamps, Cell, 78 (1994) 877-887.

[156] P.J. Lau, R.D. Kolodner, Transfer of the MSH2.MSH6 complex from proliferating cell nuclear antigen to mispaired bases in DNA, The Journal of biological chemistry, 278 (2003) 14-17.

[157] R.E. Georgescu, L. Langston, N.Y. Yao, O. Yurieva, D. Zhang, J. Finkelstein, T. Agarwal, M.E. O'Donnell, Mechanism of asymmetric polymerase assembly at the eukaryotic replication fork, Nature structural & molecular biology, 21 (2014) 664-670.

[158] M.M. Ghodgaonkar, F. Lazzaro, M. Olivera-Pimentel, M. Artola-Boran, P. Cejka, M.A. Reijns, A.P. Jackson, P. Plevani, M. Muzi-Falconi, J. Jiricny, Ribonucleotides misincorporated into DNA act as strand-discrimination signals in eukaryotic mismatch repair, Molecular cell, 50 (2013) 323-332.

[159] S.A. Lujan, J.S. Williams, A.R. Clausen, A.B. Clark, T.A. Kunkel, Ribonucleotides are signals for mismatch repair of leading-strand replication errors, Molecular cell, 50 (2013) 437-443.

[160] S. Allen-Soltero, S.L. Martinez, C.D. Putnam, R.D. Kolodner, A saccharomyces cerevisiae RNase H2 interaction network functions to suppress genome instability, Molecular and cellular biology, 34 (2014) 1521-1534.

[161] T.A. Kunkel, D.A. Erie, Eukaryotic Mismatch Repair in Relation to DNA Replication, Annual review of genetics, (2015).

[162] F. Li, G. Mao, D. Tong, J. Huang, L. Gu, W. Yang, G.M. Li, The histone mark H3K36me3 regulates human DNA mismatch repair through its interaction with MutSalpha, Cell, 153 (2013) 590-600.

[163] F. Li, L. Tian, L. Gu, G.M. Li, Evidence that nucleosomes inhibit mismatch repair in eukaryotic cells, The Journal of biological chemistry, 284 (2009) 33056-33061.

[164] S. Javaid, M. Manohar, N. Punja, A. Mooney, J.J. Ottesen, M.G. Poirier, R. Fishel, Nucleosome remodeling by hMSH2-hMSH6, Molecular cell, 36 (2009) 1086-1094.

[165] L.Y. Kadyrova, E.R. Blanko, F.A. Kadyrov, CAF-I-dependent control of degradation of the discontinuous strands during mismatch repair, Proceedings of

the National Academy of Sciences of the United States of America, 108 (2011) 2753-2758.

[166] B. Schopf, S. Bregenhorn, J.P. Quivy, F.A. Kadyrov, G. Almouzni, J. Jiricny, Interplay between mismatch repair and chromatin assembly, Proceedings of the National Academy of Sciences of the United States of America, 109 (2012) 1895-1900.

[167] C. Rayssiguier, D.S. Thaler, M. Radman, The barrier to recombination between Escherichia coli and Salmonella typhimurium is disrupted in mismatch-repair mutants, Nature, 342 (1989) 396-401.

[168] P. Shen, H.V. Huang, Effect of base pair mismatches on recombination via the RecBCD pathway, Molecular and general genetics, 218 (1989) 358-360.

[169] K. Myung, A. Datta, C. Chen, R.D. Kolodner, SGS1, the Saccharomyces cerevisiae homologue of BLM and WRN, suppresses genome instability and homeologous recombination, Nature genetics, 27 (2001) 113-116.

[170] R.M. Spell, S. Jinks-Robertson, Examination of the roles of Sgs1 and Srs2 helicases in the enforcement of recombination fidelity in Saccharomyces cerevisiae, Genetics, 168 (2004) 1855-1865.

[171] C.D. Putnam, T.K. Hayes, R.D. Kolodner, Specific pathways prevent duplication-mediated genome rearrangements, Nature, 460 (2009) 984-989.

[172] M. Honda, Y. Okuno, S.R. Hengel, J.V. Martin-Lopez, C.P. Cook, R. Amunugama, R.J. Soukup, S. Subramanyam, R. Fishel, M. Spies, Mismatch repair protein hMSH2-hMSH6 recognizes mismatches and forms sliding clamps within a D-loop recombination intermediate, Proceedings of the National Academy of Sciences of the United States of America, 111 (2014) E316-325.

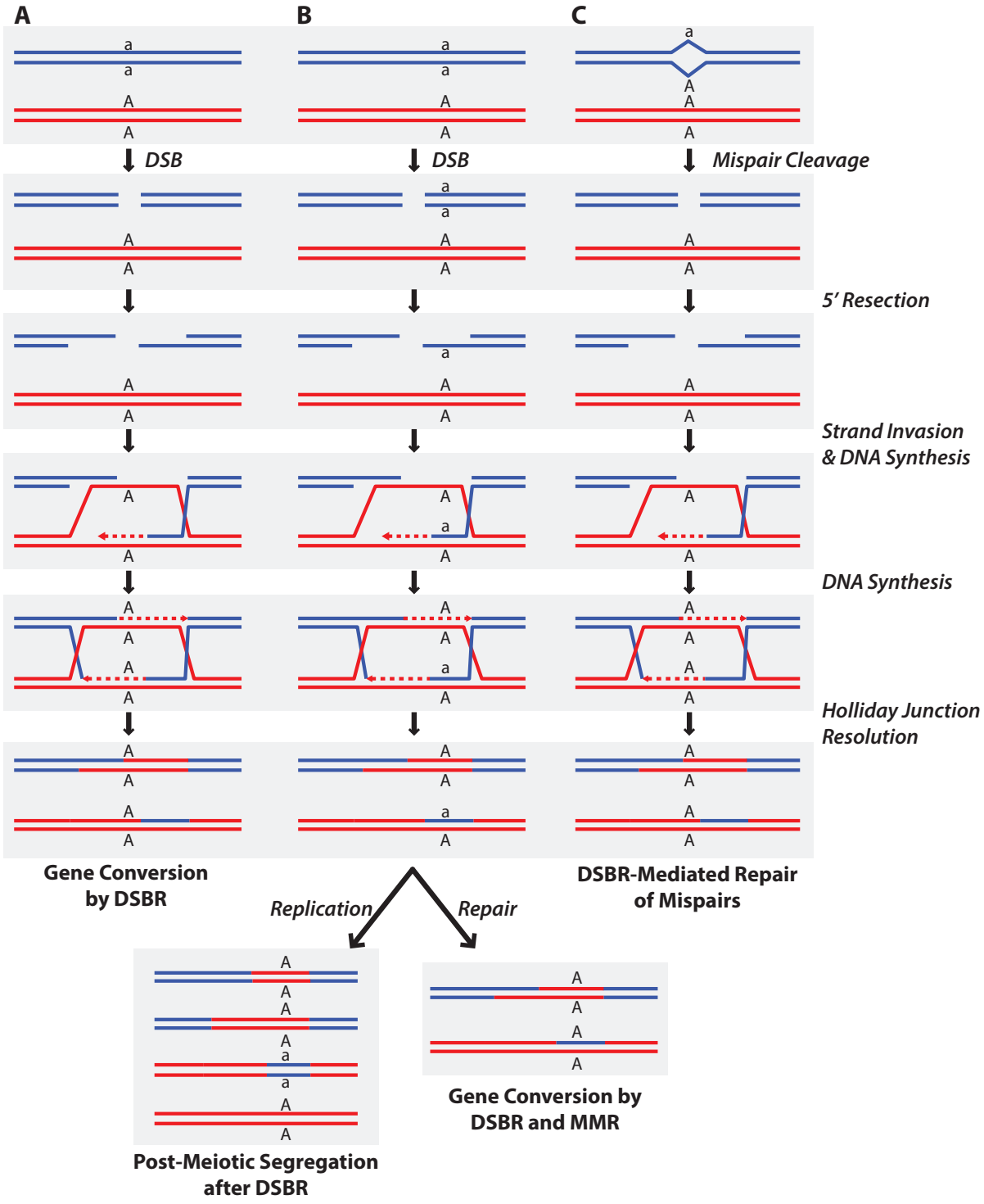
[173] L. Foiry, L. Dong, C. Savouret, L. Hubert, H. te Riele, C. Junien, G. Gourdon, Msh3 is a limiting factor in the formation of intergenerational CTG expansions in DM1 transgenic mice, Human genetics, 119 (2006) 520-526.

[174] W.J. van den Broek, M.R. Nelen, D.G. Wansink, M.M. Coerwinkel, H. te Riele, P.J. Groenen, B. Wieringa, Somatic expansion behaviour of the (CTG)n repeat in myotonic dystrophy knock-in mice is differentially affected by Msh3 and Msh6 mismatch-repair proteins, Human molecular genetics, 11 (2002) 191-198.

[175] S.A. Martomo, P.J. Gearhart, Somatic hypermutation: subverted DNA repair, Current opinion in immunology, 18 (2006) 243-248.

[176] G.M. Li, The role of mismatch repair in DNA damage-induced apoptosis, Oncology research, 11 (1999) 393-400.

[177] D. Fu, J.A. Calvo, L.D. Samson, Balancing repair and tolerance of DNA damage caused by alkylating agents, Nature reviews. Cancer, 12 (2012) 104-120.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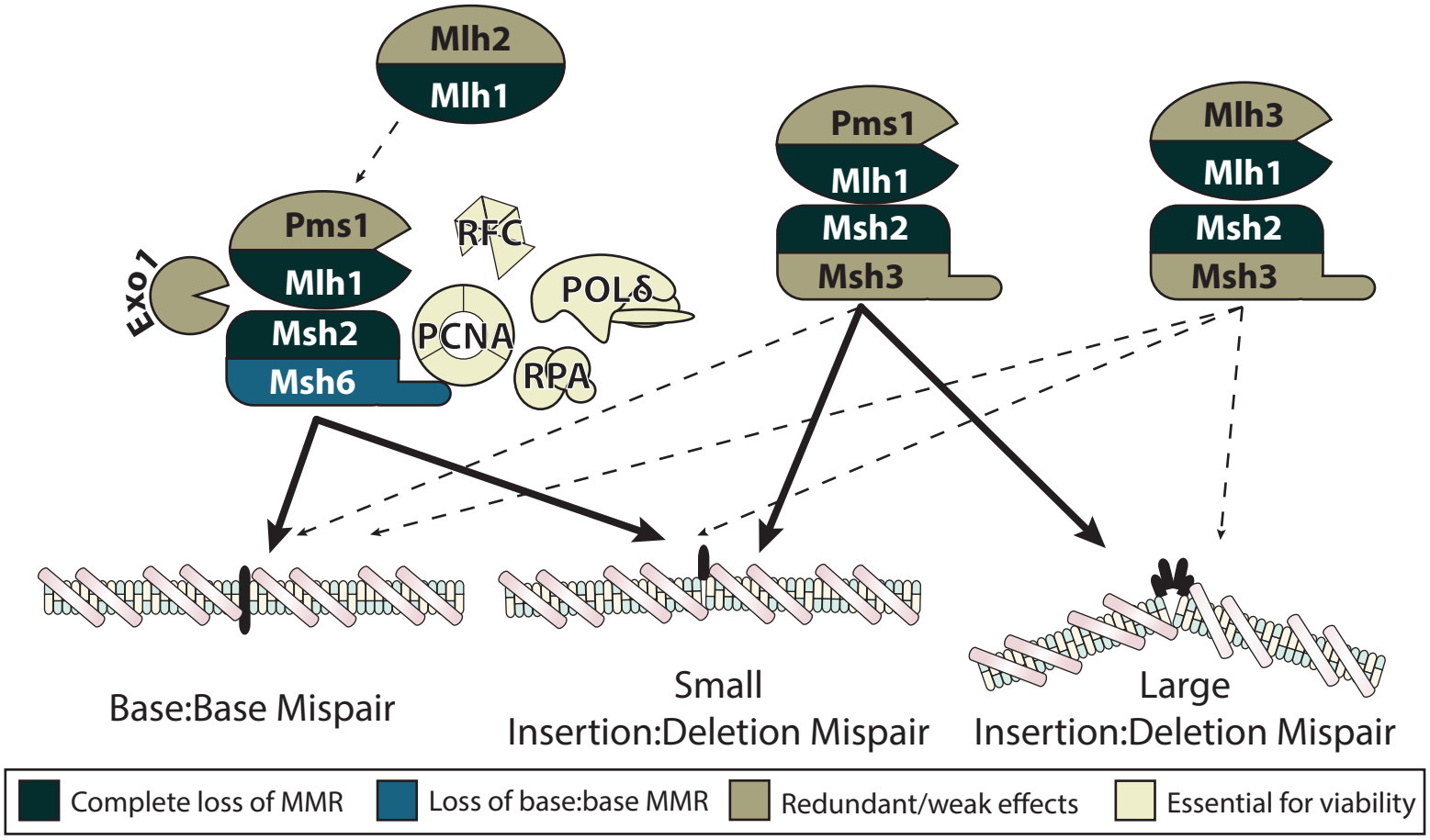
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Base:Base Mispair
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Insertion:Deletion Mispair
Large
Insertion:Deletion Mispair
Complete loss of MMR
Loss of base:base MMR
Redundant/weak effects
Essential for viability