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Chromatin Remodeling and Mismatch Repair: Access and Excision

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Abstract

DNA mismatch repair (MMR) increases replication fidelity and genome stability by correcting DNA polymerase errors that remain after replication. Defects in MMR result in the accumulation of mutations and lead to human tumor development. Germline mutations in MMR cause the hereditary cancer syndrome, Lynch syndrome. After replication, DNA is reorganized into its chromatin structure and wrapped around histone octamers. DNA MMR is thought to be less efficient in recognizing and repairing mispairs packaged in chromatin, in which case MMR must either compete for access to naked DNA before histone deposition or actively move nucleosomes to access the mispair. This article reviews studies into the mechanistic and physical interactions between MMR and various chromatin-associated factors, including the histone deposition complex CAF1. Recent Xenopus and *Saccharomyces cerevisiae* studies describe a physical interaction between Msh2 and chromatin-remodeling ATPase Fun30/SMARCAD1, with potential mechanistic roles for SMARCAD1 in moving histones for both mispair access and excision tract elongation. The RSC complex, another histone remodeling complex, also potentially influences excision tract length. Deletion mutations of *RSC2* point to mechanistic interactions with the MMR pathways. Together, these studies paint a picture of complex interactions between MMR and the chromatin environment that will require numerous additional genetic, biochemical, and cell biology experiments to fully understand. Understanding how these pathways interconnect is essential in fully understanding eukaryotic MMR and has numerous implications in human tumor formation and treatment.

Keywords

Mismatch repair; Genome instability; Chromatin; Fun30; SMARCAD1; CAF1

1. DNA Mismatch Repair and Cancer

DNA mismatch repair (MMR) is a postreplicative repair pathway responsible for removing base-base mispairs and small insertions or deletions not corrected by the proofreading

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abilities of the replicative DNA polymerases (1, 2). The MMR process in eukaryotic organisms includes recognition of the mispair, followed by DNA endonuclease nicking of the daughter strand, excision of the daughter strand past the mispaired base pair and gap-filling by a DNA polymerase (Figure 1) (3, 4). In model organisms and mammalian cell culture studies, mutations in genes disrupting any of these steps leads to an increase in mutation accumulation. Defects in MMR are now known to underlie the development and progression of human tumors, presumably due to the high level of accumulating mutations in MMR deficient cells (5–8).

Lynch syndrome is a hereditary cancer predisposition syndrome that increases the risks of colon, endometrial, ovarian, stomach, and other cancers (9–12). An autosomal dominant pattern of gastric and colon cancer development was originally described in the early 1900s in a large extended family. Later, the discovery of additional families by Henry Lynch and others eventually led to the identification of what was then termed hereditary nonpolyposis colon cancer (hnpcc) and later renamed Lynch syndrome (13). In 1993 it was realized that colon cancers from Lynch syndrome families harbored altered lengths in sequences that contained simple repetitive “microsatellite” sequences (13). Microsatellite instability (MSI) is a hallmark of MMR defects (14). Later the same year, Richard Kolodner cloned human Msh2 and linked Msh2 to Lynch Syndrome (5), followed by Bert Vogelstein’s group finding additional Msh2 mutations in Lynch Syndrome families (15). Other MMR genes, notably Mlh1, Pms2, and Msh6 have since been shown to be mutated in Lynch Syndrome families (16, 17).

Microsatellite instability and somatic alterations in MMR genes are frequently seen in sporadic tumors of numerous origins (12, 18). In addition to MMR gene mutations, the promoter region of Mlh1 can by hypermethylated, thus silencing Mlh1 and leading to an MSI-high tumor phenotype (19). Defects in MMR, in addition to being tumor-promoting, also lead to resistance to common chemotherapeutics such as 5-fluorouracil, cisplatin and carboplatinum, and temozolomide (20). Although the mechanisms are not well understood, a functional MMR pathway is required for the recognition of certain DNA lesions and the downstream signaling that ultimately results in apoptotic cell death (21). For example, MMR is required for the recognition of O^6-methylguanine mispaired with a T that can occur after treatment with an alkylating agent,.

Despite the overall resistance to chemotherapy, colon cancers with MSI-high phenotypes have a better clinical prognosis in general (22, 23). One theory is that this is due to the more favorable immune system engagement with these tumors (24). With the dawn of immunotherapy, MMR deficient/MSI-high tumor types seem to respond particularly well to anti-PD checkpoint inhibitors in general. This enhanced response is thought to be due to the increased generation of neo-antigens due to a high mutational burden (25). As advances continue to be made in immunotherapy and chemotherapy and as our ability to sequence human tumors increases, continuing to understand the detailed mechanisms of the MMR pathway is critical to our future success in treating patients.

Many details of how the MMR pathway works in eukaryotes remain to be discovered. Among these are the emerging studies into how MMR functions in a chromatin environment

and how chromatin factors interplay with MMR processes. MMR is believed to follow behind the progressing replication fork, correcting errors left over by the proofreading replicative DNA polymerases, Polδ and Polε. MMR proteins should be closely coordinated with the replication machinery in order to accomplish this. However, a comprehensive understanding of this interplay between the two pathways is not fully worked out. Among the outstanding questions is whether or not MMR occurs primarily in the naked DNA environment occurring immediately after replication, or if mispair recognition also occurs in an environment containing partially or fully reconstituted nucleosomes. Furthermore, whether MMR has to accommodate a variety of chromatin states at different steps of MMR or in different chromosomal locations or cellular contexts is still a question, and if so, how are these states managed. MMR also has multiple mechanistic steps following the initial recognition of the mispair (Figure 1), and it is unclear if different steps in the process require different interactions with histones or chromatin-associated factors. A further understanding of these interactions among MMR, chromatin-associated factors, and the chromatin landscape is paramount to fully understanding the fundamental mechanisms of MMR.

2. DNA mismatch repair and chromatin assembly

MMR is spatially and temporally linked with DNA replication (26, 27). The MMR mispair recognition complexes, Msh2-Msh6 and Msh2-Msh3, bind to the mispair and can undergo a conformational change in a mispair- and ATP-dependent manner into a sliding-clamp formation that allows diffusion along the DNA (28, 29). Msh2-Msh6 physically binds to the replicative clamp loader, PCNA, through an unstructured tether on Msh6 (30, 31). This interaction can be visualized *in vivo* as Msh2 co-localizing with labeled replication factories (26, 32). Loss of the PCNA-Msh6 interaction results in a 10–15% decrease in MMR in the *Saccharomyces cerevisiae* system as a single mutation; however, in the absence of *EXO1* MMR becomes absolutely dependent on the Msh6-PCNA (26, 33, 34). Together this suggests at least a portion of MMR *in vivo* requires a physical link to replication (26, 33, 34). MMR has been thought to preferentially take place during S-phase (27) when DNA is actively replicating. Experiments in yeast using cell cycle restricted MMR genes showed that MMR could only take place within a short window after a region has been replicated (27). Artificially restricting Msh6 expression to the G2/M cell cycle phase by fusing Msh6 with a fragment of *CLB2* under the control of the cell cycle-regulated *CLB2* cyclin promoter, resulted in the loss of ability for MMR to correct errors in a frameshift reversion assay that replicates in mid-S-phase. However, the movement of the assay to a late replicating region allowed repair by G2/M expressed Msh6, but not S-phase expressed Msh6 (27).

Also taking place behind the replication fork is the deposition of nucleosomes and the packaging of DNA into a higher-order chromatin structure. This process is carried out in part by the histone chaperone, chromatin assembly factor 1 (CAF1) that binds to PCNA and deposits newly synthesized $(H3\text{-}H4)_2$ tetramers onto DNA (35). The full nucleosome is later formed by the addition of H2A and H2B dimers. With both of these two processes, repair and histone deposition, taking place shortly behind the replication fork, a natural question is how these two processes influence each other. This question has been addressed by several studies utilizing elegant *in vitro* MMR assays that have been augmented with nucleosome

deposition systems, reviewed below. Human MutSα (Msh2-Msh6) easily recognizes mispairs *in vitro* on naked DNA (36). The addition of nucleosome octamers added to a DNA substrate with a mispair and nucleosome positioning sites showed that MutSα binds to mispairs between nucleosome octamers, but binds to mispairs incorporated into nucleosomes to a lesser degree (37). In *in vitro* MMR assays utilizing human cell extracts, excess MMR-associated degradation of the nicked strand can be observed for a mispair containing plasmid substrate incubated with cytosolic HeLa cell extract (38). The same MMR associated degradation was not seen in a nuclear HeLa extract. Further investigation led to the identification of CAF1 as the nuclear factor that suppresses excess MMR provoked degradation of the discontinuous strand (38). CAF1 nick-dependent deposition of nucleosomes is hypothesized to limit the sliding of the MutS-MutL incision complex away from the mispair, thus limiting excess degradation (38). This observation that histones can block MMR protein movement on DNA is similar to the observed block to the MutSα sliding clamp movement seen by Li et al. (37). Together, this raises the hypothesis that the MMR reaction must take place before DNA is packaged into nucleosomes or otherwise displace nucleosomes.

When CAF1 nucleosome assembly is incorporated into *in vitro* MMR assays, MutSα can repress nucleosome assembly at that mispair and adjacent to the mispair in the plasmid substrate (38–40). A functional MMR pathway slows the overall deposition of nucleosomes (38–40). This slowing of nucleosome assembly seems to allow efficient MMR, as the addition of a CAF1 nucleosome assembly reaction, with or without the ASF1 chaperone, did not diminish MMR reactions *in vitro* (39, 40). This was true regardless of whether the *in vitro* reactions contained Exo1 or were dependent on DNA pol δ-mediated strand displacement (40). The authors also carried out reactions in which DNA Polε carried out the gap-filling step. In the DNA Pol ε reactions, CAF1 and ASF1-H3-H4 actually led to slightly enhanced MMR capacity in the *in vitro* assay. This was hypothesized to be due in part to the suppression of excessive degradation of the discontinuous strand (40). It is worth noting that the results of *in vitro* studies, at least in part, depend on the specifics of histone deposition in the system, the strength of the nucleosome-DNA interaction, and the concentration of proteins present. While Li et al. observe a block to MutSα sliding clamp movement by established nucleosomes, alternately, Javaid et al. show that a Msh2-Msh6 sliding clamp can dissociate a nucleosome from DNA in a mispair dependent manner (41). This dissociation is enhanced by H3 acetylation, suggesting interplay could depend on context.

In addition to an important role in the repair of replication-associated errors, MMR protein interactions with nucleosome deposition components may also play a role in response to cytotoxic lesions that are recognized and processed by the MMR pathway. In the *Saccharomyces cerevisiae* system, the deletion of either the *CAC1* or *CAC2* subunits of yeast CAF1resulted in an increased sensitivity to SN1-type alkylating agent MNNG (42). The authors propose this is through the excessive MMR-dependent degradation of the discontinuous strand that then results in additional conversion of the nicked DNA to doublestrand breaks (42). Deletion of Rtt106, also involved in replication-associated nucleosome deposition, does not alter MNNG sensitivity like *CAF1* depletion, nor does the deletion of the non-replication associated histone chaperone, HIR (42). This may indicate redundancy *in vivo* as compared to the *in vitro* system or may be an inherent difference in

how MMR and histone deposition interact in relation to replication-associated mispairs versus irreparable cytotoxic mispairs. Recent *in vivo* genome-wide analysis using the newly developed NMP-seq method in *Saccharomyces cerevisiae* showed that base excision repair of alkylation-induced N7-methylguanine and N3-methyladenine is faster in nucleosome-depleted regions. Furthermore, alkylation-induced mutations accumulate at sites of strong nucleosome positioning (43). Overall, the toxicity and mutagenicity of alkylating agents are likely closely tied with chromatin structure, regardless of whether the lesion is an MMR or base excision repair substrate.

This coordination between nucleosome assembly and MMR is likely to be facilitated by direct protein-protein interactions between the MMR protein MutSα and components of the nucleosome deposition machinery. Both CAF1 and Msh6 have known interactions with PCNA (33, 34, 44, 45). PCNA is a critical component of the MMR pathway not only for gap filling but in upstream steps, including the stimulation of the MutLα endonuclease (46–49). Interactions with PCNA seem to be especially critical in Exo1-independent pathways of MMR requiring highly active Mlh1-Pms1 (Pms2 in human) endonuclease activity (50). One possibility is that PCNA interacting with MutSα may prevent CAF1 binding, which may explain the ability of MMR to prevent nucleosome deposition near the mispair. CAF1 also directly binds MutSα, and this binding is enhanced during S-phase, in part due to enhanced Msh6 expression during S-phase (39). MutSα is capable of pulling down purified ASF1-H3-H4, although the interaction seems to be with the histone components and not the ASF chaperone components (42). Understanding how these multiple protein interactions work together is essential for fully understanding MMR occurring behind the replication fork.

3. DNA mismatch repair and recruitment by histone modifications

MMR components have also been found to physically interact with histone modifications. Human Msh6 has a Pro-Trp-Trp-Pro (PWWP) motif in the unstructured N-terminal domain facilitating binding to the H3K36me histone mark (51). This interaction between human MutSα and chromatin modifications that peak in G1/S-phase and diminish in G2, suggests enhanced recruitment of MMR activity during S-phase of the cell cycle. A previous special edition of *DNA Repair* reviewed this work (52). The common cancer-driving H3G34V/R/D mutations in the H3 subunit block the interaction with MutSα (53). Recently it has also been suggested that the MutSα interaction with the chromatin H3K36me3 marks may also play a role in promoting MMR activity at sites of transcription (54).

4. DNA mismatch repair and chromatin remodeling

In a recent study, initially designed to characterize the interaction between Msh2 and Exonuclease 1 (Exo1), a novel Msh2 interaction peptide motif (SHIP box) was newly identified (55–57). This Msh2 interaction peptide had similar characteristics to the previously identified Mlh1 interaction peptide motif (MIP box) given that it consisted primarily of two bulky hydrophobic residues in a region of Exo1 predicted to be unstructured and largely hydrophilic. However, unlike the MIP box in which two phenylalanine residues are directly adjacent to each other, the SHIP box motif consists of a phenylalanine or tyrosine spaced with one unconserved amino acid in between (57, 58).

These motifs are notable in that while the intrinsically unstructured C-terminal tail of Exo1 is poorly conserved throughout evolution in length and primary amino acid sequence, the MIP and SHIP box motifs themselves are well conserved from fungi through humans, although their location within the C-terminal region varies. The MIP box motif was originally identified as the interaction site between base excision repair protein, Ntg2, and Mlh1, but has been identified as a common interaction motif in at least two other proteins, Exo1 and Sgs1(58–60). Based on the idea that the MIP box is a shared mode of binding to Mlh1 by several proteins, a bioinformatic search was performed to identify if the SHIP box may also be a shared mode of Msh2 binding and to identify potential novel SHIP box containing proteins. Using a positionspecific scoring matrix (PSSM) to measure similarity to the SHIP box motif and IUPRED analysis to determine predicted disorder, the entire yeast proteome was analyzed for potential SHIP box containing proteins (57). A subset of proteins was prioritized for validation by yeast two-hybrid assay based on high PSSM/IUPRED score and/or previous association with DNA metabolism or repair. From this study, two *Saccharomyces cerevisiae* SHIP box containing proteins were confirmed, the DNA polymerase ε subunit, Dpb3, and chromatin remodeling factor, Fun30. Binding of these protein partners with Msh2 is mediated by the SHIP box motif, as the mutation of the F/Y amino acids to alanine disrupted the *in vivo* binding (57).

Fun30, and its human homolog, SMARCAD1, is an SNF-2 family nucleosome remodeler with ATPase activity that has been shown to have roles in the control of histone turn over during replication, control of translation, and resection during homologous recombination (61–65). It is also implicated in the restoration of heterochromatin after replication and is known to bind PCNA (66, 67). In the study of *FUN30* in homologous recombination end resection, *FUN30* was initially selected for study out of a *Saccharomyces cerevisiae* screen that looked for deletion mutations that resulted in a lower frequency of homologous recombination promoted gene integration (65). Further experiments show that the deletion of *FUN30* inhibited long-range resection by Exo1 5kb to 28kb away from the induced double-strand break, and both the helicase and ATPase functions of the protein were required for long-range resection. The deletion of other nucleosome remodeling complexes implicated in resection, *RSC2,* and to a lesser extent *INO80,* when combined with deletion of *FUN30* had a synergistic effect on long-range resection (65). The excision step of MMR can be carried out through either exonuclease-dependent or exonuclease-independent mechanisms (4). Exo1 performs the excision step in exonuclease-dependent MMR. The binding of Msh2 to both Fun30 and Exo1 suggests this interaction could play a role in controlling excision, similar to the role of Fun30 in homologous recombination.

Unsurprisingly, a single mutation deletion of *FUN30* did not affect MMR capacity *in vivo* as measured by frameshift reversion assays. In genetic backgrounds that eliminate Exo1-independent repair through a pol30-K217E mutation (49), *fun30Δ* caused a small but statistically significant increase in mutation rate (57). Similar to what was seen in assays for homologous recombination excision tract length, the deletion of *RSC2*, seems to have a synergistic effect with the deletion of *FUN30.* These data point to a need for nucleosome remodeling during Exo1 excision based MMR, in at least a subset of repair reactions. Given the relatively small change in mutation rate, it can be hypothesized that either extensive excision and chromatin remodeling is not required in a majority of MMR reactions, or that

additional chromatin remodeling factors play redundant roles in the process. Additional studies *in vivo* and in *in vitro* reconstitution reactions will be required to determine if Fun30 and Rsc2 change Exo1 processivity or excision tract length in the presence of nucleosomes. In a genetic background in which Exo1-independent repair occurs (*exo1Δ* mutation), *rsc2Δ* also increases the mutation rate. The *rsc2Δfun30Δ* double mutant, however, has a lower mutation rate than the single rsc2Δ (57). This data suggest the role of chromatin remodelers in Exo1-independent repair, carried out by either DNA polymerase δ strand displacement (68) or successive rounds of endonuclease nicking (48, 49) may be more complicated and require further studies to comprehend fully. Roles for Fun30 discovered in yeast, such as in resection after a double-strand break, seem to translate to the human homolog SMARCAD1 (64). It is likely that the same will be true for the Msh2-SMARCAD1 interaction. Human peptide analysis of potential SHIP box containing proteins using the PSSM and IUPRED analysis pipeline led to the identification of human Exo1-SHIP boxes along with a high score for the potential SMARCAD1 SHIP box. Phylogenetic analysis indicates that this motif is well conserved from yeast Fun30 to human SMARCAD1 (57).

In support of the idea that the Msh2-Fun30/SMARCAD1 interaction is significant throughout evolution, Msh2 and *Xenopus* SMARCAD1 physically interact, and SMARCAD1 depletion changes nucleosome exclusion around mispairs in extract-based repair assays (69). In *Xenopus* nucleoplasmic egg extracts, plasmids are supercoiled due to HIRA deposition of histones. A mispair in the plasmid disrupts plasmid supercoiling leading nucleosomes to be excluded in a 1kb region around the mispair. By immunodepleting the extracts of either Msh2-Msh6 or Mlh1-Pms2, it was shown that this nucleosome exclusion phenotype is Msh2-Msh6 dependent. Furthermore, the addition of Msh2 to the immunodepleted extract could displace preassembled nucleosomes (69).

Xenopus Msh2 and SMARCAD1 also directly bind. By linking the mispair containing plasmid to biotin, the authors were able to pull down associated proteins and look for proteins enriched in the presence of a mispair. SMARCAD1 and the FACT subunits, spt16 and ssrp1, were increased when a mispair was present. SMARCAD1 was confirmed to co-immunoprecipitate with Msh2 (69). Both this study and the above *Saccharomyces cerevisiae* study independently identified a Fun30/SMARCAD1-Msh2 interaction through unbiased approaches, strengthening the evidence that this is a significant interaction. In the *Xenopus* extract system, the depletion of SMARCAD1 led to more supercoiling around the mispair. An ATPase mutant of SMARCAD1 also resulted in increased supercoiling in the region around the mispair, suggesting that the ATPase function of SMARCAD1 is required (69). The presence of SMARCAD1 also facilitated MMR in the condition where nucleosomes were preassembled around the mispair in a *Xenopus* nucleoplasmic extract (69). This study brings up the possibility that Fun30/SMARCAD1 is an MMR accessory factor that may allow both access to naked DNA and control excision in Exo1-dependent MMR. What contribution redundant chromatin remodeling factors play is yet to be clarified.

While chromatin marks may help recruit MMR to chromatin during S-phase, carrying out efficient MMR in the context of chromatin is a challenge the cell must overcome, whether that is through inhibition of chromatin deposition or the movement of already established nucleosomes. The studies reviewed above clearly point to important and conserved

interactions between Msh2-Msh6/MutSα and chromatin metabolism proteins. (Figure 2) Detailed studies into how these pathways interplay with each other are required if we want to fully understand what effect they have on genome stability and the development of human tumors.

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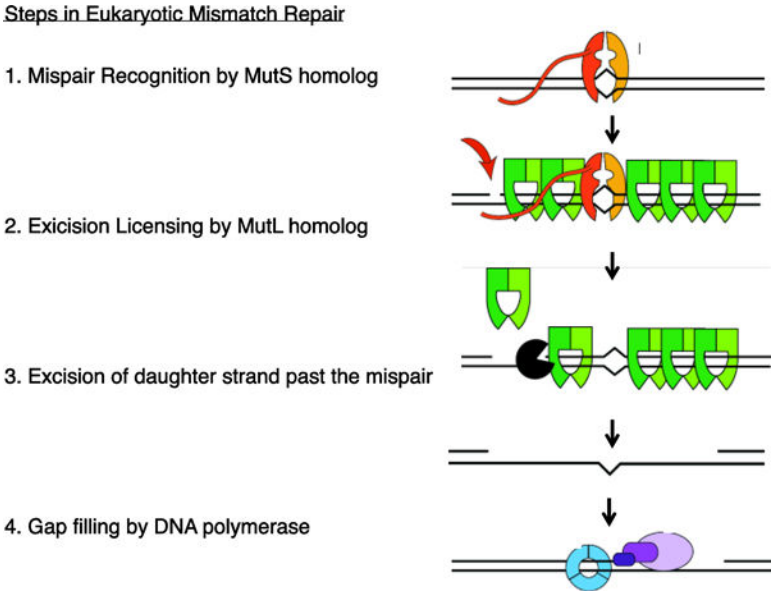

Figure 1. Steps in Eukaryotic Mismatch Repair.

MMR occurs after replication to repair base-base mispairs and small insertion/deletion loops. Eukaryotic MMR occurs using a set of common steps: mispair recognition by the MutS homologs, recruitment of MutL homologs containing endonuclease activity, recruitment of Exo1 exonuclease and excision of the daughter strand, and gap filling by the replicative DNA polymerases.

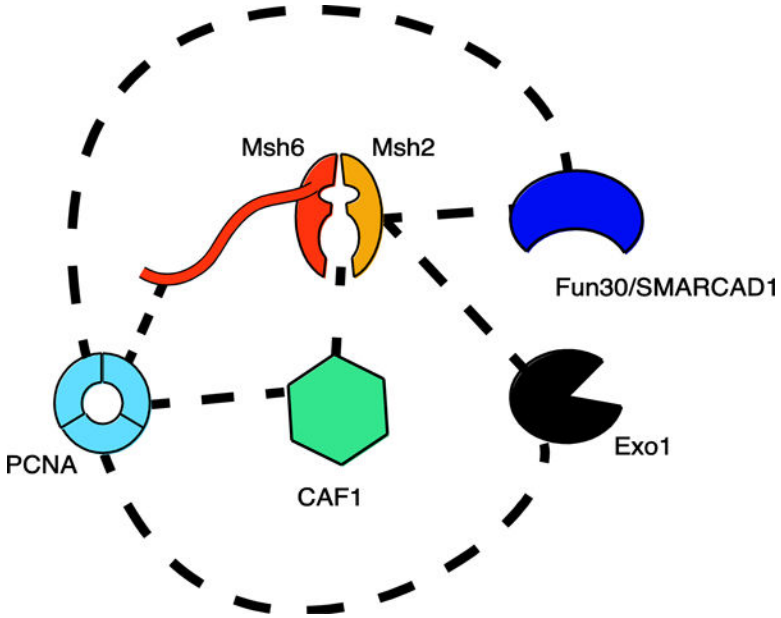

Figure 2. Reported interactions between Msh2-Msh6 (MutSα) and chromatin remodeling proteins.

Dashed lines indicated reported physical interactions between proteins in either *Saccharomyces cerevisiae*, *Xenopus*, or Humans. There is a high level of physical interaction between the MutS homologs and chromatin modulating proteins, with PCNA being a common interacting protein. Not all interactions are confirmed in all species. Yeast Exo1 lacks the PCNA PIP-box that is contained in human Exo1.