

SURVEY AND SUMMARY

The UvrD helicase and its modulation by the mismatch repair protein MutL

Steven W. Matson^{1,2,3,*} and Adam B. Robertson¹¹Department of Biology, ²Curriculum in Genetics and Molecular Biology and ³Program in Molecular and Cellular Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA

Received March 25, 2006; Revised June 11, 2006; Accepted June 14, 2006

ABSTRACT

UvrD is a superfamily I DNA helicase with well documented roles in excision repair and methyl-directed mismatch repair (MMR) in addition to poorly understood roles in replication and recombination. The MutL protein is a homodimeric DNA-stimulated ATPase that plays a central role in MMR in *Escherichia coli*. This protein has been characterized as the master regulator of mismatch repair since it interacts with and modulates the activity of several other proteins involved in the mismatch repair pathway including MutS, MutH and UvrD. Here we present a brief summary of recent studies directed toward arriving at a better understanding of the interaction between MutL and UvrD, and the impact of this interaction on the activity of UvrD and its role in mismatch repair.

INTRODUCTION

Methyl-directed mismatch repair (MMR) is the primary pathway for correcting replication errors and errant recombination events in *Escherichia coli* (1–8) making a functional MMR pathway essential for ensuring the informational integrity of the chromosome, genomic stability and an acceptable cellular mutation rate. Functionally homologous repair systems, directed towards the repair of base pair mismatches as well as small insertions and deletions, also exist in eukaryotic organisms [for reviews see (4,5,8,9)]. Importantly, homologues of the bacterial MutS and MutL proteins have been identified in every cellular organism studied to date, including yeast and humans, suggesting that the basic strategy used to repair base pair mismatches and insertion/deletion loops is highly conserved. Although the *in vivo* signal for strand discrimination and the mechanism of damage-containing strand excision are uncertain in eukaryotes, mismatch recognition and repair involving the MutS and MutL homologues appears to be very

similar to the bacterial system. The fundamental importance of a functional mismatch repair pathway is underscored by the fact that, in humans, defects in mismatch repair result in genomic instability that can lead to certain types of cancer, especially hereditary colon cancer (10–15).

The primary components of the bacterial MMR pathway have been uncovered in a series of elegant biochemical and genetic studies, and include the mutator proteins (MutL, MutS, MutH and UvrD/MutU), several exonucleases, including ExoI, ExoVII, RecJ and ExoX, DNA polymerase III, single-stranded DNA (ssDNA) binding protein, DNA ligase and the Dam methylase (16–23). These proteins act in concert, after passage of the replication fork, to correct base pair mismatches and small insertion–deletion mutations. The complete mismatch repair pathway has been reconstituted *in vitro* (24) and the sequence of events in the mismatch repair process has been well described using this *in vitro* system [for reviews see (4,7,25)]. Nonetheless, mechanistic issues, particularly those surrounding the role of ATP binding/hydrolysis and the role of various protein–protein interactions, remain unresolved.

A current model of MMR in *E.coli* (4,7,25–27) in posits a set of carefully orchestrated steps (Figure 1). Mismatch recognition is accomplished by MutS, which recognizes and binds to the base pair mismatch (23,28) in a process that requires ATP. The bacterial MutS protein has been crystallized in a complex with a mismatch (28) and shown to interact asymmetrically with the DNA. One subunit in the homodimer interacts with the mismatch [or looped out base(s)] while the other interacts non-specifically with the DNA. This is followed by the binding of MutL to the MutS–DNA complex to form a ternary complex containing both proteins and DNA (29–31). Both MutL and MutS are functional as homodimers [heterodimers in eukaryotic cells (8)] and both proteins are capable of binding and hydrolyzing ATP (26,27,32,33). The rate of ATP hydrolysis catalyzed by MutS is faster than that of MutL and the molecular role of ATP binding and hydrolysis by these two proteins is under investigation. The MutS–MutL complex then communicates with MutH bound at a transiently hemi-methylated

*To whom correspondence should be addressed. Department of Biology, CB# 3280, Coker Hall, University of North Carolina, Chapel Hill, NC 29577-3280, USA. Tel: +1 919 962 0005; Fax: +1 919 962 1625; Email: smatson@bio.unc.edu

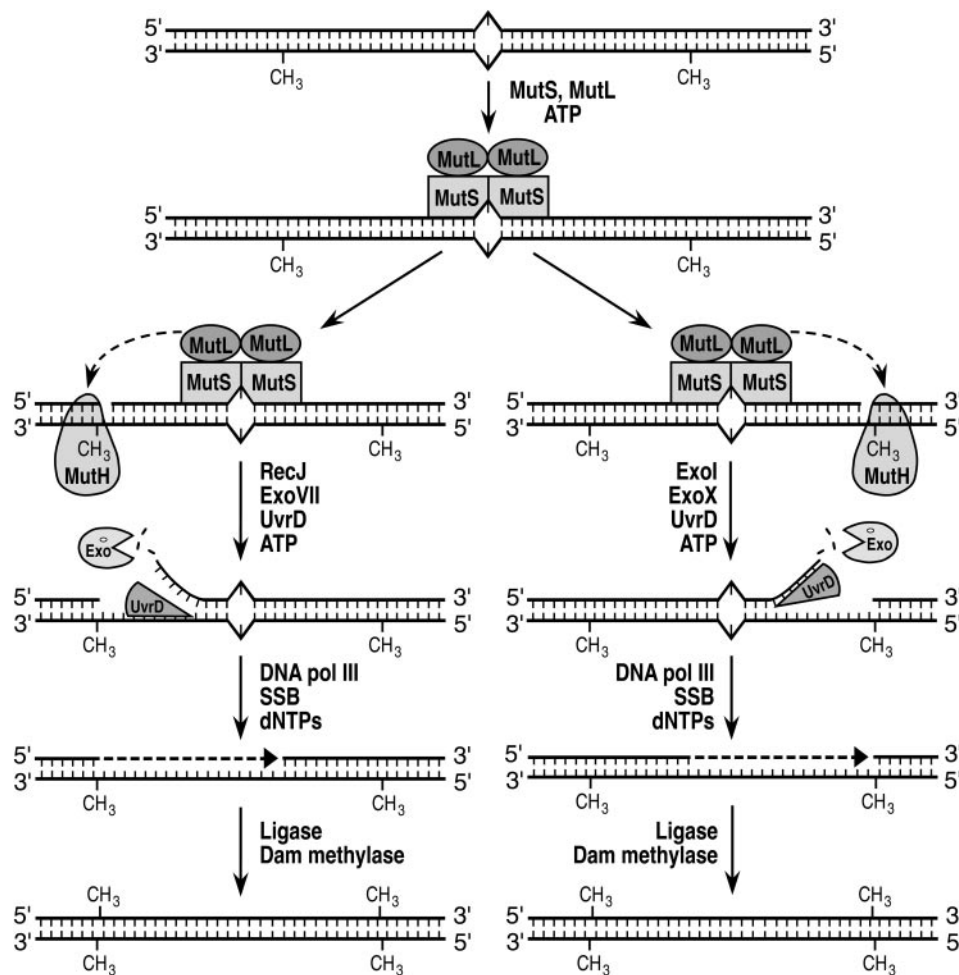


Figure 1. Schematic model of MMR in *E. coli*. The base–base mismatch is recognized by the MutS homodimer and a ternary complex containing DNA, MutL and MutS is formed in a reaction requiring ATP. This complex communicates with the nearest hemi-methylated d(GATC) sequence in some manner that is not yet fully understood and stimulates nicking on the unmethylated (nascent) DNA strand by MutH to provide strand discrimination. The nearest hemi-methylated d(GATC) site may be located on either side of the mismatch providing MMR with bidirectional capability. UvrD is loaded by MutL and unwinds the duplex DNA beginning at the nick and extending toward the mismatch. The damaged DNA strand is degraded by one of several ssDNA exonucleases involved in mismatch repair depending on the polarity of the required strand resection. The ssDNA gap is stabilized by the binding of ssDNA binding protein and the gap is filled by DNA polymerase III. The resulting nick is sealed by DNA ligase to restore the integrity of the DNA stand and the d(GATC) is fully methylated by Dam methylase.

d(GATC) site. Precisely, how this communication occurs is still a matter of debate. There are currently three models for which there is some experimental support. In one model the MutS–MutL complex is proposed to loop out the DNA, actively searching for the nearest d(GATC) methylation site either 5' or 3' of the mismatch (18,34). In the second model MutS binds the mismatch in an ADP bound state which provokes an ADP–ATP exchange. This produces a conformational change that induces MutS to act as a sliding clamp capable of leaving the mismatch by diffusion. This process occurs iteratively and the MutS–MutL complexes interact with and activate the MutH endonuclease (35). The third model proposes that MutS remains bound at or near the mismatch with communication with MutH bound at the hemi-methylated site involving DNA bending (36). While details regarding the nature of this communication are still unresolved, it is clear that once the nearest hemi-methylated d(GATC) site is found, the MutS–MutL complex activates the latent endonuclease activity associated with MutH to

generate a nick in the DNA on the unmethylated (nascent) DNA strand (19,21). This provides discrimination of the parental strand from the daughter strand and directs the subsequent repair event to the nascent DNA strand. Activation of the latent endonuclease activity of MutH is presumably accomplished by MutL, since MutL has been shown to be capable of stimulating this reaction in the absence of MutS (37). Once the nascent DNA strand has been nicked, DNA helicase II (UvrD) unwinds the DNA beginning at the nick and moving toward the mismatch (38) and one of several ssDNA exonucleases (ExoI, ExoVII, ExoX and RecJ), with an appropriate polarity, degrades the nascent DNA strand as it is being unwound (16,38). Resection of the damaged DNA continues to a point that is, on average, about 100 bp past the mismatch (18) and ssDNA binding protein binds and stabilizes the ssDNA template until DNA polymerase III is recruited to fill the gap. The resulting nick is sealed by DNA ligase, completing the repair process and restoring the integrity of the DNA (24).

The use of the nearest hemi-methylated d(GATC) as the site of MutH-directed incision provides MMR with a bidirectional capability since this site could be located on either the 5' side or the 3' side of the mismatch (17,18). However, UvrD unwinds DNA with a specific 3' to 5' polarity (39,40). Therefore, UvrD must be loaded on the appropriate DNA strand in order to unwind toward the mismatch. It is also important to note that the d(GATC) site nearest the base pair mismatch may be 1–2 kb away from the mismatch (21,41) although, repair efficiency decreases as a function of increasing distance between the d(GATC) initiation site and the mismatch. This fact has important consequences for understanding the unwinding event catalyzed by the modestly processive UvrD helicase. The signal indicating sufficient DNA has been unwound/degraded to complete the repair process is not known.

The role of MutL protein, characterized as the master regulator of mismatch repair, remains to be completely defined on a mechanistic level. The protein was originally purified, using a biochemical complementation assay, as an essential component for partially reconstituted mismatch repair in cell extracts lacking MutL (31). Subsequent experiments demonstrated its interaction with MutS at a mismatch (30,31,42), and the solved crystal structure of the N-terminal domain of MutL (43,44) demonstrated an ATP binding/hydrolysis fold common to the GHKL group (gyrase/Hsp90/histidine-kinase/MutL) of ATP hydrolyzing enzymes (45). Purified MutL catalyzes a very slow ATP hydrolysis reaction that is stimulated by the presence of ssDNA and is essential for MMR (43,44,46). In addition, MutL has been shown to interact with MutH and activate the hemi-methylated d(GATC)-directed nicking reaction catalyzed by MutH (19,37), as well as stimulating unwinding catalyzed by UvrD (38,47,48). Thus, MutL is capable of interacting with and modulating the activity of many of the key protein players in mismatch repair.

The stimulation of MutL-catalyzed ATP hydrolysis by the addition of DNA has prompted an investigation of the DNA binding properties of MutL. Several groups have demonstrated that MutL binds to both ssDNA and double-stranded DNA (dsDNA) (30,48–51), while others report that MutL does not bind DNA (52) or suggest that binding to DNA may be irrelevant to its function (35). Of particular interest is the fact that MutL specifically stimulates the duplex DNA unwinding reaction catalyzed by UvrD (38,47,48). The unwinding activity of Rep protein (37% identical to UvrD) is also enhanced by MutL, but to a significantly lower extent (47). In addition, on a nicked, circular heteroduplex DNA substrate, MutL and MutS together activated UvrD-catalyzed unwinding while there was no detectable enhancement of unwinding by Rep helicase (38). Since the stimulation of unwinding by MutL is specific to UvrD it is likely that the mechanism of stimulation by MutL is through a direct protein–protein interaction. Moreover, the interaction between MutL and UvrD is likely to be critical for the repair process.

UvrD exhibits modest processivity as a DNA helicase (40–50 bp) (53–55) making this protein an interesting choice for the helicase responsible for the unwinding event in MMR. UvrD is also the helicase responsible for the unwinding event associated with excision repair (56–59), which requires

unwinding of a short 12–13 base long oligonucleotide well within the limits of the processivity of UvrD. MMR, on the other hand, can require the unwinding of up to 1–2 kb of DNA, which is substantially in excess of the reported processivity of UvrD. In some manner, not fully understood, MutL is able to modulate the unwinding reaction catalyzed by UvrD to allow the unwinding of long duplex regions in the context of MMR.

Little is known about the mechanism by which the UvrD-catalyzed unwinding reaction is enhanced by MutL. This issue has received some attention recently (26,48,51) and some important details of the reaction have been described. For example, on a nicked, circular molecule containing a mismatch, MutS, MutL and UvrD initiate unwinding at the nick site and begin helix opening in the direction toward the mismatch. This reaction requires all three protein components and the presence of a mismatch (38). Moreover, while MutL dramatically stimulates the unwinding reaction catalyzed by UvrD, MutL does not increase its rate of ATP hydrolysis (60). Thus, UvrD becomes a more efficient helicase in the presence of MutL unwinding more base pairs of DNA per ATP hydrolysis event than in the absence of MutL. Experiments with model substrates support a mechanism in which MutL directs the productive loading of UvrD onto a DNA substrate. We have proposed that the loading of UvrD is an iterative process such that multiple molecules of UvrD accumulate on the substrate to increase the rate of progressive unwinding and to facilitate the unwinding of long duplex regions using a helicase with modest processivity. This model suggests that (i) an interaction between MutL and UvrD is required for MMR, (ii) the ATP binding/hydrolysis activity of MutL, which is known to be essential for MMR (46), is likely to play some role in modulating the interaction between MutL and UvrD and (iii) DNA binding by MutL is essential for stimulation of UvrD-catalyzed unwinding and, therefore, MMR. Each of these questions will be addressed, in turn, in the following discussion of the modulation of the activity of UvrD by MutL.

MutL interacts with UvrD

An interaction between MutL and UvrD was demonstrated several years ago using the yeast two-hybrid system and confirmed in biochemical studies (37). The yeast two-hybrid analysis revealed a direct interaction between UvrD and MutL, and deletion analysis indicated that critical residues for the interaction with UvrD were located between amino acids 397 and 438 and after amino acid 559 of the 615 amino acid MutL protein. It was not possible to localize the region on UvrD responsible for binding to MutL using this approach since removal of either the N- or C-terminal ends of UvrD eliminated the two-hybrid interaction. The MutL interaction surface on UvrD remains to be defined.

The recent solution of the structure for the C-terminal domain of MutL (29) has allowed a prediction of the interaction site between UvrD and MutL. Based on the two-hybrid results reported by Hall *et al.* (60) and the folding of C-terminal domain of MutL, it is likely that UvrD interacts with a region on MutL involving a portion of the disordered linker between the N- and C-terminal domains of MutL that lies nearest the C-terminal domain and the MutL C-terminus.

Consistent with this assignment is the fact that the N-terminal domain of MutL, which does not include the disordered linker region, fails to stimulate the unwinding reaction catalyzed by UvrD (29). However, the N-terminal domain has been suggested to interact with UvrD based on crosslinking studies (29). The amount of crosslinked protein observed in this study was extremely low and may not reflect a significant interaction. We have shown that deletion of the last 20 amino acids of MutL significantly reduces the MutL–UvrD interaction in both physical and functional assays (S.R. Pattishall and S.W. Matson, unpublished data). However, an alternate interpretation of the structural data for the C-terminal domain (61) suggests that the C-terminal end is directly involved in dimer formation and that a truncation lacking the C-terminal 20 amino acids should be a monomer instead of a dimer. Our recent experiments with this truncation mutant indicate that the protein is, in fact, a dimer as originally suggested (29). This conclusion was arrived at using gel exclusion chromatography at an ionic strength that is nearly physiological (200 mM) and analytical ultracentrifugation. We suggest that UvrD is likely to directly interact with the linker region and the C-terminal end of MutL. These two regions are located near one another in the 3D model of MutL (29). Importantly, the MutL Δ C20 deletion mutant maintains ~60% of the ATPase activity associated with MutL, binds DNA, interacts with MutS and stimulates MutH-directed nicking in a reaction that is dependent on both MutS and the presence of a mismatch base pair (S.R. Pattishall and S.W. Matson, unpublished data). Thus, the protein is properly folded and capable of making all the required protein–protein interactions necessary for mismatch repair. However, MutL Δ C20 fails to stimulate UvrD in helicase activity assays and does not complement a *mutL* deletion in genetic complementation studies. Thus, the C-terminus of MutL is essential for MMR *in vivo* as recently reported (61) and the interaction between MutL and UvrD is essential for loading UvrD onto the repair substrate and is essential for full functionality of the MMR pathway.

MutL stimulates DNA unwinding by UvrD

We and others have shown that MutL dramatically stimulates the unwinding activity of UvrD (38,47,48). Previously, we proposed that MutL loads UvrD productively onto the DNA for unwinding but does not clamp UvrD on the DNA during the unwinding reaction (48). These experiments, performed using model DNA substrates and in the absence of other mismatch repair proteins, also suggested that loading of UvrD by MutL was likely to be an iterative process. The unwinding reaction catalyzed by UvrD demonstrates a limited processivity of 40–50 bp (54) yet DNA repair tracts can be up to 1 kb in length. However, the processivity of UvrD as a translocase is significantly higher (2400 \pm 600 nt) (62). Thus, the iterative loading of UvrD by MutL may address the issue of how to produce long repair tracts using a helicase with limited processivity.

The first indication that MutL acted to load UvrD onto DNA came from DNA binding studies showing that the addition of MutL increased the affinity of UvrD for DNA. Electrophoretic gel mobility shift experiments revealed that UvrD, in the presence of AMP-PNP, formed a weak

complex with ssDNA that dissociated during the course of electrophoresis and was difficult to detect. In the presence of MutL, a supershifted MutL–UvrD–ssDNA complex was formed that was more stable than the UvrD–ssDNA complex indicating that MutL + UvrD formed a specific complex with a greater affinity for ssDNA than UvrD alone (48).

Based on these results two possibilities were considered; (i) MutL increased the rate of UvrD association with the DNA or (ii) MutL decreased rate of dissociation of UvrD from ssDNA. An increased rate of association of UvrD with ssDNA would be reflected as increased loading of UvrD onto model helicase substrates. In single turn-over experiments with a 20 bp partial duplex substrate, preincubation of UvrD and MutL with the DNA resulted in a stimulation of the amount of product produced reflecting an increase in the amount of productively loaded UvrD. The concentration at which MutL was half saturating for the burst phase amplitude of UvrD-catalyzed DNA unwinding (40.2 \pm 9.7 nM) was similar to the K_D of MutL for binding partial duplex DNA (24.3 \pm 0.7 nM). This result was interpreted to indicate that binding of MutL to DNA was important for its role in stimulating the unwinding reaction catalyzed by UvrD and that MutL was stimulating the loading of UvrD onto the DNA substrate.

If MutL decreased the UvrD dissociation rate one could envision MutL functioning as a clamp, keeping UvrD tethered to ssDNA and effectively increasing its processivity. Results from two experiments have suggested this is not the case. First, unwinding assays with a 148 bp blunt duplex substrate, well in excess of the measured processivity of UvrD, failed to detect increased unwinding in the presence of MutL after the addition of a ssDNA trap. Second, single turn-over assays with a 92 bp partial duplex DNA compared with a 20 bp partial duplex DNA also indicated that MutL was not acting to alter the processivity of UvrD. Under identical conditions, a smaller fraction of 92 bp partial duplex molecules were unwound in comparison to 20 bp molecules. If MutL were acting to increase the processivity of UvrD then the same fraction of substrate should have been unwound in each case. Moreover, the degree of stimulation was similar on both substrates suggesting that stimulation was independent of substrate length and, therefore, likely a loading phenomenon.

Increased loading of UvrD by MutL was also investigated using long DNA substrates to model the lengths of DNA substrates likely to be encountered *in vivo*. Data from multiple turn-over helicase reactions with a 750 bp blunt duplex substrate and an 851 bp partial duplex substrate support the notion that MutL loads UvrD onto DNA, and further suggest that loading by MutL is likely to be an iterative process. Unwinding of the two long substrates was generally described by a burst phase followed by a steady-state phase. The burst phase for these reactions reflects unwinding by those UvrD molecules that were pre-loaded onto the DNA substrate. Comparison of the unwinding kinetics exhibited by UvrD on the 750 bp blunt duplex DNA in the presence or absence of MutL clearly showed that in the absence of MutL there was no burst phase. This reflects an inability of UvrD alone to efficiently pre-load on blunt duplex substrates as shown previously (54). In the experiments using the 750 bp blunt duplex and 851 bp partial duplex substrates the increased

productive loading of UvrD is likely to be continuous over the entire course of the unwinding reaction. Considering the reported processivity for UvrD (40–50 bp), completion of unwinding of these longer substrates and detection of the significant burst phase in the unwinding assay with the 750 bp blunt duplex DNA requires multiple binding events by UvrD.

Based on these results a model was proposed to explain the stimulation of UvrD-catalyzed DNA unwinding by MutL (Figure 2). The first step is the loading of UvrD onto the DNA. In the presence of MutL this is enhanced due to an increased affinity of the UvrD–MutL complex for DNA. After it is loaded UvrD begins to unwind the duplex. In the presence of MutL additional molecules of UvrD are loaded behind the leading molecule of UvrD and the high concentration of UvrD may increase the overall rate of UvrD-catalyzed unwinding. Eventually, the leading molecule of UvrD will dissociate from the duplex since UvrD translocates through duplex DNA an average of ten steps (40–50 bp) before dissociating (54). In the case of UvrD alone, when

the leading molecule dissociates the partially unwound duplex can reanneal and the whole process must start over. On the other hand, in the presence of MutL multiple UvrD molecules have been loaded onto the duplex and the DNA does not reanneal; the additional UvrD molecules translocate along the ssDNA template and continue unwinding. This is consistent with the observation that UvrD is considerably more processive as a translocase moving along ssDNA (62) than as a DNA helicase.

As noted above, MutL-catalyzed ATP hydrolysis is required for MMR (46,51). However, the rate of MutL-catalyzed ATP hydrolysis is slow and it seems unlikely this fuels active translocation along the DNA lattice suggesting that ATP hydrolysis may play another role (30,35). Perhaps stimulation of the UvrD-catalyzed unwinding reaction requires the hydrolysis of ATP by MutL. If this were the case, then the MutL-catalyzed ATP hydrolysis requirement in MMR might be explained by the requirement for ATP hydrolysis in loading UvrD to begin resection of the damaged DNA strand. Such a requirement would be consistent with the previous characterization of MutL as a molecular matchmaker (63).

Using a MutL point mutant, MutL-E29A, that binds but does not hydrolyze ATP, Robertson *et al.* (64) have demonstrated that MutL-catalyzed ATP hydrolysis is not required for MutL-dependent stimulation of the UvrD unwinding reaction. In fact, the unwinding reaction catalyzed by UvrD on both a partial duplex substrate and a blunt end duplex substrate was significantly more efficient in the presence of MutL-E29A; maximal stimulation of the unwinding reaction occurred at much lower concentrations of MutL-E29A than observed with the wild-type protein. This was not the result of higher affinity binding of MutL-E29A to the DNA substrate since binding to both a partial duplex ligand and a ssDNA ligand by MutL-E29A was similar to that of the wild-type protein. Importantly, it is the ATP-bound form of MutL that is specifically responsible for stimulating UvrD (64). A second MutL point mutant, MutL-D58A, which does not bind ATP does not stimulate the unwinding reaction catalyzed by UvrD. Thus, the ATP-bound form of MutL stimulates UvrD-catalyzed unwinding while the ATP free form of MutL does not stimulate UvrD-catalyzed unwinding.

Ban and Yang (44) have shown that binding of ATP causes the N-terminal domain of MutL to dimerize and perhaps this causes the protein to clamp onto the DNA substrate. In the context of MMR this would occur at the nick generated by MutH (Figure 3). This may provide a loading platform for UvrD from which helicase molecules could be continuously loaded onto the DNA substrate as long as MutL remained clamped on the DNA. In the absence of ATP hydrolysis (i.e. with MutL-E29A) MutL remains bound and loads multiple molecules of UvrD resulting in a very dramatic stimulation of UvrD-catalyzed unwinding at low concentrations of MutL. If this is the case then MutL-catalyzed ATP hydrolysis acts to regulate the loading of UvrD onto the MMR intermediate.

This result has important implications for our understanding of the process of MMR. First, it serves to further refine our understanding of the role of ATP binding and hydrolysis catalyzed by MutL in MMR. It has been established

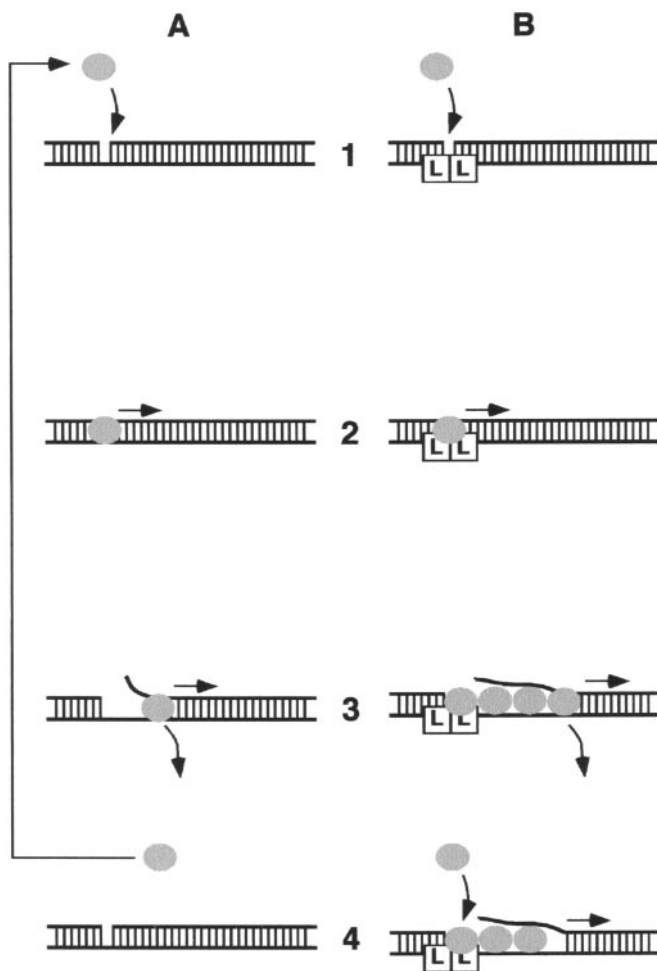


Figure 2. Model for the mechanism of MutL-stimulated unwinding catalyzed by UvrD. A model for unwinding by UvrD (A) or UvrD + MutL (B) on a nicked DNA substrate is shown. Details for the model are discussed in the text. Step 1 is preloading; step 2 is with one molecule of UvrD loaded; step 3 is unwinding and step 4 is after dissociation of a molecule of UvrD. This figure is reproduced from (48).

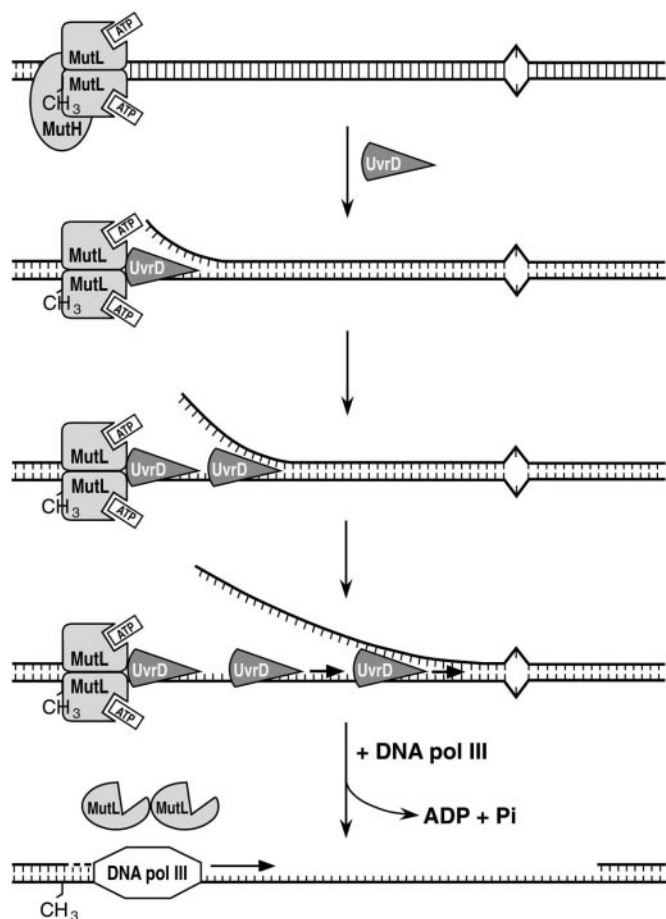


Figure 3. A model depicting the loading of UvrD by MutL in MMR. A mismatch in the DNA is shown. The nick is introduced by MutH on the unmethylated DNA strand at a hemi-methylated d(GATC) site. A dimer of MutL, in the ATP-bound form, is shown loading UvrD onto the nicked DNA. The UvrD loading event ensures that the helicase is loaded onto the correct strand to translocate in the 3' to 5' direction toward the mismatch. The ATP-bound form of MutL is active in loading UvrD. ATP hydrolysis by MutL is not required to actively load UvrD. MutL loads multiple molecules of UvrD to ensure the unwinding of repair tracks that may be in excess of 1 kb length. We hypothesize that ATP hydrolysis by MutL releases the protein from the DNA and stops the loading of UvrD.

previously that ATP binding but not hydrolysis by MutL is required for interaction with MutS (42) and it has been demonstrated that the ATP-bound form of MutL stimulates the latent endonuclease reaction associated with MutH (37). Thus, MutL-catalyzed ATP hydrolysis is required after strand incision and the beginning stage of strand resection. Second, these results suggest the possibility that MutL-catalyzed ATP hydrolysis regulates the amount of UvrD loaded onto the DNA substrate. In the absence of MutL-catalyzed ATP hydrolysis the unwinding step (catalyzed by UvrD) may be uncoupled from the resection step (catalyzed by an exonuclease) such that the exonuclease responsible for removing the unwound damage-containing nascent strand is unable to keep up with the advancing helicase. If this were the case then repair events might not be properly completed due to this uncoupling and there would be an increase in mutation rate as was observed when *mutL-E29A* was substituted for *mutL* (51,64).

The biological advantage of using MutL as an auxiliary factor that increases the initiation rate and progressive unwinding rate by UvrD in mismatch repair is clear. The mismatch repair pathway requires, in some instances, the unwinding of long tracts of DNA (22). In the absence of MutL the low processivity of UvrD seems inconsistent with the long repair patch lengths in light of the observed efficiency of mismatch repair. Therefore, the iterative loading of UvrD by MutL would increase both the rate and the efficiency of the reaction, and enable UvrD to unwind the long tracts required in this pathway despite its relatively low intrinsic processivity. In addition, it is known that UvrD unwinds a nicked DNA molecule poorly presumably due to poor binding of this substrate (65,66). The use of MutL as a specific loading factor at the nick created by MutH overcomes this limitation and ensures the loading of UvrD on the biological substrate.

Loading of UvrD by MutL would also explain the ability of UvrD to unwind toward the mismatch. The mismatch repair reaction has bidirectional capability since the hemi-methylated d(GATC) site may be located on either side of the mismatch [see Figure 1; (17,18)]. However, UvrD unwinds duplex DNA with a specific polarity (39,53,62,67). Therefore, in order for UvrD to unwind toward the mismatch it must be loaded onto the appropriate strand to unwind with its known polarity. If MutL functions to load UvrD on the DNA, this provides a mechanism to load UvrD exclusively on the appropriate strand. This would prevent UvrD from unwinding non-specifically in both directions as was observed for UvrD-catalyzed unwinding on nicked substrates in the absence of mismatch repair proteins (65,66).

MutL-mediated loading of UvrD requires DNA binding by MutL

The model for MutL-stimulated unwinding of DNA by UvrD presented above predicts that the DNA binding activity of MutL is essential. The role of DNA binding by MutL in the process of mismatch repair has been debated in the literature for the last 14 years. While there is considerable evidence suggesting that MutL binds DNA (44,48,50), there is also evidence to the contrary (52), and recent experiments have suggested that DNA binding may be an artifact of *in vitro* experiments (35). Here we summarize new data obtained using biochemical and genetic assays to characterize a MutL point mutant (MutL-R266E) in an effort to evaluate the biological importance of DNA binding by MutL. Taken together, the *in vivo* and *in vitro* results strongly suggest that MutL must bind DNA as part of the MMR process (49).

MutL-R266E has been described as having reduced DNA binding affinity (42,44). The crystal structure of dimeric MutL (43) positions arginine 266 facing inward and a previous report (29) has suggested that it could interact with the negatively-charged backbone of DNA. Thus, arginine 266 may be a primary amino acid involved in recognizing the DNA through electrostatic interactions. Purified MutL-R266E retains the biochemical properties of wild-type MutL that do not involve DNA binding. These properties include: (i) basal ATP hydrolysis, (ii) an ability to interact with MutH, MutS, and UvrD and (iii) the ability to dimerize. These results demonstrate that the mutant protein retains both

its tertiary structure and its dimeric form. In addition, MutL-R266E is able to catalyze the hydrolysis of ATP. However, the properties dependent upon DNA binding are severely compromised in this mutant (49). The basal ATPase activity exhibited by the mutant protein was not stimulated by the addition of ssDNA, whereas the basal ATPase of wild-type MutL was significantly stimulated by the addition of ssDNA. The mutant protein was also significantly reduced in its ability to stimulate the unwinding reaction catalyzed by UvrD, although at high concentrations, it was able to stimulate UvrD on a partial duplex DNA substrate. With these characteristics in mind, it is not unexpected that the MutL-R266E exhibits a strong mutator phenotype. We conclude, based on both genetic and biochemical data, that DNA binding by MutL is critical for MMR consistent with the model for MutL-stimulated unwinding catalyzed by UvrD proposed above.

We note there is significant support for this conclusion in studies involving MMR in eukaryotes. In yeast, it has been demonstrated that MutL homologues must bind DNA in order for MMR to occur (68). Point mutations in the yeast homologues (PMS1-K328E and MLH1-R273E, R274E), similar to the point mutation analyzed here, increase the mutation frequencies and rates *in vivo*.

SUMMARY AND PERSPECTIVE

A model for the role of MutL in *E.coli* mismatch repair, based on the data reviewed above, can be summarized as follows. In the first step, a mismatch generated during replication is recognized by the MutS dimer and a MutS–MutL interaction mediates communication with MutH bound at the nearest hemi-methylated d(GATC) site. Precisely how MutL facilitates the communication between the mismatched base pair and MutH bound at the hemi-methylated d(GATC) site is still uncertain. After nicking on the nascent strand by MutH, UvrD is loaded by MutL and initiates unwinding at the nick toward the mismatch. The mismatch repair system displays bidirectional capability and correction of a mismatched base can be directed by a hemi-methylated d(GATC) sequence on either the 5' or 3' side of the error (17,18). However, UvrD unwinds exclusively in a 3' to 5' direction with respect to the bound DNA strand (39,40). Therefore, the capacity for bidirectional unwinding from a nick necessitates the ability of UvrD to load onto either one of the DNA strands. We and others (29,38,48) have suggested that one role for the interaction between MutL and UvrD is to orient the helicase, by loading it onto the appropriate DNA strand at the nicked d(GATC) site, so that it unwinds in the proper direction for mismatch excision. This would explain the *in vitro* observations of Modrich and colleagues (18,38) that UvrD preferentially unwinds toward the error in the context of the mismatch repair system and is consistent with the fact that MutL directly interacts with UvrD. Studies using model DNA substrates indicate that the ATP-bound form of MutL stimulates UvrD-catalyzed unwinding presumably by loading UvrD onto the DNA substrate. We hypothesize that MutL remains bound to the DNA, continuously loading molecules of UvrD, until one or both of the bound ATP molecules are hydrolyzed to ADP and Pi. After an ATP

hydrolysis fueled conformational change, MutL is released from the DNA and stops loading UvrD. If this is the case, then one role of the MutL ATPase is to act as a molecular switch to facilitate initiation of the excision event which involves activating the MutH endonuclease and loading UvrD onto the DNA. The processivity of UvrD-catalyzed unwinding of duplex DNA has been estimated at 40–50 bp in one study (54) and ~250 bp in another study (55). However, repair tracts *in vivo* can be up to 1 kb in length (21,41), and we have demonstrated significant unwinding of duplex DNA substrates in excess of 850 bp in the presence of MutL. In a recent study by Fischer *et al.* (62) the processivity of UvrD was determined to be 2400 + 600 bases as the protein translocates along ssDNA. We suggest that the multiple UvrD molecules loaded onto the DNA by MutL are able to unwind 1–2 kb of duplex DNA in the following way: as one molecule of UvrD dissociates from the DNA (due to its intrinsic processivity as a helicase), a second molecule of UvrD takes its place, and so on, allowing unwinding to continue uninterrupted. This helps to ensure that, no matter what the distance between the initiating nick and the mismatch, adequate DNA is unwound by UvrD to complete repair of the mismatch.

Given its multiple roles in MMR, MutL is likely to be the master coordinator in the mismatch repair reaction. The interaction of MutL with the three mismatch repair proteins MutS, MutH and UvrD and with DNA is critical for the proper 3D arrangement of these proteins, as well as for the appropriate sequential timing for each event in the repair reaction. Precisely how MutL accomplishes all of these goals remains to be elucidated. However, the results reviewed here begin to explain the mechanism by which MutL stimulates UvrD-catalyzed DNA unwinding, demonstrate the importance of MutL binding to DNA and indicate that ATP hydrolysis by MutL is not required for the loading of UvrD. In addition, they serve to better position the site of MutL-catalyzed ATP hydrolysis within the mismatch repair pathway. It is now clear that MutL-catalyzed ATP hydrolysis is required after strand excision has begun. It is becoming more important to identify those regions of MutL responsible for the interaction with the other components of the mismatch repair pathway to understand how MutL functions in all of its roles.

ACKNOWLEDGEMENTS

The authors thank Susan Whitfield for help in preparation of the artwork and the Matson laboratory for useful discussions. Work from the authors' laboratory was supported by a grant from the National Institutes of Health to S.W.M. Funding to pay the Open Access publication charges for this article was provided by National Institutes of Health Grant GM33476 to Steven W. Matson.

Conflict of interest statement. None declared.

REFERENCES

1. Modrich, P. and Lahue, R. (1996) Mismatch repair in replication fidelity, genetic recombination, and cancer biology. *Annu. Rev. Biochem.*, **65**, 101–133.

2. Modrich,P. (1991) Mechanisms and biological effects of mismatch repair. *Annu. Rev. Genet.*, **25**, 229–253.
3. Modrich,P. (1989) Methyl-directed DNA mismatch correction. *J. Biol. Chem.*, **264**, 6597–6600.
4. Kunkel,T.A. and Erie,D.A. (2005) DNA mismatch repair. *Annu. Rev. Biochem.*, **74**, 681–710.
5. Harfe,B.D. and Jinks-Robertson,S. (2000) DNA mismatch repair and genetic instability. *Annu. Rev. Genet.*, **34**, 359–399.
6. Rayssiguier,C., Thaler,D.S. and Radman,M. (1989) The barrier to recombination between *Escherichia coli* and *Salmonella typhimurium* is disrupted in mismatch-repair mutants. *Nature*, **342**, 396–401.
7. Schofield,M.J. and Hsieh,P. (2003) DNA mismatch repair: molecular mechanisms and biological function. *Annu. Rev. Microbiol.*, **57**, 579–608.
8. Jiricny,J. (2006) The multifaceted mismatch-repair system. *Nature Rev. Mol. Cell. Biol.*, **7**, 335–346.
9. Kolodner,R. (1996) Biochemistry and genetics of eukaryotic mismatch repair. *Genes Dev.*, **10**, 1433–1442.
10. Edelmann,L. and Edelmann,W. (2004) Loss of DNA mismatch repair function and cancer predisposition in the mouse: animal models for human hereditary nonpolyposis colorectal cancer. *Am. J. Med. Genet. C. Semin. Med. Genet.*, **129**, 91–99.
11. Charames,G.S. and Bapat,B. (2003) Genomic instability and cancer. *Curr. Mol. Med.*, **3**, 589–596.
12. Li,G.M. (2003) DNA mismatch repair and cancer. *Front. Biosci.*, **8**, d997–1017.
13. Fishel,R. (2001) The selection for mismatch repair defects in hereditary nonpolyposis colorectal cancer: revising the mutator hypothesis. *Cancer Res.*, **61**, 7369–7374.
14. Jiricny,J. and Nystrom-Lahti,M. (2000) Mismatch repair defects in cancer. *Curr. Opin. Genet. Dev.*, **10**, 157–161.
15. Guerrette,S., Acharya,S. and Fishel,R. (1999) The interaction of the human MutL homologues in hereditary nonpolyposis colon cancer. *J. Biol. Chem.*, **274**, 6336–6341.
16. Burdett,V., Baitinger,C., Viswanathan,M., Lovett,S.T. and Modrich,P. (2001) In vivo requirement for RecJ, ExoVII, ExoI, and ExoX in methyl-directed mismatch repair. *Proc. Natl Acad. Sci. USA*, **98**, 6765–6770.
17. Cooper,D.L., Lahue,R.S. and Modrich,P. (1993) Methyl-directed mismatch repair is bidirectional. *J. Biol. Chem.*, **268**, 11823–11829.
18. Grilley,M., Griffith,J. and Modrich,P. (1993) Bidirectional excision in methyl-directed mismatch repair. *J. Biol. Chem.*, **268**, 11830–11837.
19. Au,K.G., Welsh,K. and Modrich,P. (1992) Initiation of methyl-directed mismatch repair. *J. Biol. Chem.*, **267**, 12142–12148.
20. Welsh,K.M., Lu,A.L., Clark,S. and Modrich,P. (1987) Isolation and characterization of the *Escherichia coli* mutH gene product. *J. Biol. Chem.*, **262**, 15624–15629.
21. Lahue,R.S., Su,S.S. and Modrich,P. (1987) Requirement for d(GATC) sequences in *Escherichia coli* mutHLS mismatch correction. *Proc. Natl Acad. Sci. USA*, **84**, 1482–1486.
22. Viswanathan,M., Burdett,V., Baitinger,C., Modrich,P. and Lovett,S.T. (2001) Redundant exonuclease involvement in *Escherichia coli* methyl-directed mismatch repair. *J. Biol. Chem.*, **276**, 31053–31058.
23. Su,S.S. and Modrich,P. (1986) *Escherichia coli* mutS-encoded protein binds to mismatched DNA base pairs. *Proc. Natl Acad. Sci. USA*, **83**, 5057–5061.
24. Lahue,R.S., Au,K.G. and Modrich,P. (1989) DNA mismatch correction in a defined system. *Science*, **245**, 160–164.
25. Hsieh,P. (2001) Molecular mechanisms of DNA mismatch repair. *Mutat. Res.*, **486**, 71–87.
26. Yang,W. (2000) Structure and function of mismatch repair proteins. *Mutat. Res.*, **460**, 245–256.
27. Yang,W., Junop,M.S., Ban,C., Obmolova,G. and Hsieh,P. (2000) DNA mismatch repair: from structure to mechanism. *Cold Spring Harb. Symp. Quant. Biol.*, **65**, 225–232.
28. Sixma,T.K. (2001) DNA mismatch repair: MutS structures bound to mismatches. *Curr. Opin. Struct. Biol.*, **11**, 47–52.
29. Guarne,A., Ramon-Maiques,S., Wolff,E.M., Ghirlando,R., Hu,X., Miller,J.H. and Yang,W. (2004) Structure of the MutL C-terminal domain: a model of intact MutL and its roles in mismatch repair. *EMBO J.*, **23**, 4134–4145.
30. Schofield,M.J., Nayak,S., Scott,T.H., Du,C. and Hsieh,P. (2001) Interaction of *Escherichia coli* MutS and MutL at a DNA mismatch. *J. Biol. Chem.*, **276**, 28291–28299.
31. Grilley,M., Welsh,K.M., Su,S.S. and Modrich,P. (1989) Isolation and characterization of the *Escherichia coli* mutL gene product. *J. Biol. Chem.*, **264**, 1000–1004.
32. Lamers,M.H., Georgijevic,D., Lebbink,J.H., Winterwerp,H.H., Agianian,B., de Wind,N. and Sixma,T.K. (2004) ATP increases the affinity between MutS ATPase domains. Implications for ATP hydrolysis and conformational changes. *J. Biol. Chem.*, **279**, 43879–43885.
33. Baitinger,C., Burdett,V. and Modrich,P. (2003) Hydrolytically deficient MutS E694A is defective in the MutL-dependent activation of MutH and in the mismatch-dependent assembly of the MutS.MutL.heteroduplex complex. *J. Biol. Chem.*, **278**, 49505–49511.
34. Allen,J.D., Makhov,A., Grilley,M., Taylor,J., Thresher,R., Modrich,P. and Griffith,J.D. (1997) MutS mediates heteroduplex loop formation by a translocation mechanism. *EMBO J.*, **16**, 4467–4476.
35. Acharya,S., Foster,P.L., Brooks,P. and Fishel,R. (2003) The coordinated functions of the *E. coli* MutS and MutL proteins in mismatch repair. *Mol. Cell*, **12**, 233–246.
36. Junop,M.S., Obmolova,G., Rausch,K., Hsieh,P. and Yang,W. (2001) Composite active site of an ABC ATPase: MutS uses ATP to verify mismatch recognition and authorize DNA repair. *Mol. Cell*, **7**, 1–12.
37. Hall,M.C. and Matson,S.W. (1999) The *Escherichia coli* MutL protein physically interacts with MutH and stimulates the MutH-associated endonuclease activity. *J. Biol. Chem.*, **274**, 1306–1312.
38. Dao,V. and Modrich,P. (1998) Mismatch-, MutS-, MutL-, and helicase II-dependent unwinding from the single-strand break of an incised heteroduplex. *J. Biol. Chem.*, **273**, 9202–9207.
39. Matson,S.W. (1986) *Escherichia coli* helicase II (urvD gene product) translocates unidirectionally in a 3' to 5' direction. *J. Biol. Chem.*, **261**, 10169–10175.
40. Georgi-Geisberger,P. and Hoffmann-Berling,H. (2000) Direction of the DNA-unwinding reaction catalysed by *Escherichia coli* DNA helicase II. *Eur. J. Biochem.*, **192**, 689–693.
41. Bruni,R., Martin,D. and Jiricny,J. (1988) d(GATC) sequences influence *Escherichia coli* mismatch repair in a distance-dependent manner from positions both upstream and downstream of the mismatch. *Nucleic Acids Res.*, **16**, 4875–4890.
42. Selmane,T., Schofield,M.J., Nayak,S., Du,C. and Hsieh,P. (2003) Formation of a DNA mismatch repair complex mediated by ATP. *J. Mol. Biol.*, **334**, 949–965.
43. Ban,C. and Yang,W. (1998) Crystal structure and ATPase activity of MutL: implications for DNA repair and mutagenesis. *Cell*, **95**, 541–552.
44. Ban,C., Junop,M. and Yang,W. (1999) Transformation of MutL by ATP binding and hydrolysis: a switch in DNA mismatch repair. *Cell*, **97**, 85–97.
45. Dutta,R. and Inouye,M. (2000) GHKL, an emergent ATPase/kinase superfamily. *Trends Biochem. Sci.*, **25**, 24–28.
46. Spampinato,C. and Modrich,P. (2000) The MutL ATPase is required for mismatch repair. *J. Biol. Chem.*, **275**, 9863–9869.
47. Yamaguchi,M., Dao,V. and Modrich,P. (1998) MutS and MutL activate DNA helicase II in a mismatch-dependent manner. *J. Biol. Chem.*, **273**, 9197–9201.
48. Mechanic,L.E., Frankel,B.A. and Matson,S.W. (2000) *Escherichia coli* MutL loads DNA helicase II onto DNA. *J. Biol. Chem.*, **275**, 38337–38346.
49. Robertson,A., Pattishall,S.R. and Matson,S.W. (2006) The DNA binding activity of MutL is required for methyl-directed mismatch repair in *Escherichia coli*. *J. Biol. Chem.*, **281**, 8399–8408.
50. Bende,S.M. and Grafstrom,R.H. (1991) The DNA binding properties of the MutL protein isolated from *Escherichia coli*. *Nucleic Acids Res.*, **19**, 1549–1555.
51. Junop,M.S., Yang,W., Funchain,P., Clendenin,W. and Miller,J.H. (2003) *In vitro* and *in vivo* studies of MutS, MutL and MutH mutants: correlation of mismatch repair and DNA recombination. *DNA Repair (Amst)*, **2**, 387–405.
52. Drotschmann,K., Aronshtam,A., Fritz,H.J. and Marinus,M.G. (1998) The *Escherichia coli* MutL protein stimulates binding of vsr and MutS to heteroduplex DNA. *Nucleic Acids Res.*, **26**, 948–953.

53. Maluf,N.K., Ali,J.A. and Lohman,T.M. (2003) Kinetic mechanism for formation of the active, dimeric UvrD helicase-DNA complex. *J. Biol. Chem.*, **278**, 31930–31940.
54. Ali,J.A. and Lohman,T.M. (1997) Kinetic measurement of the step size of DNA unwinding by *Escherichia coli* UvrD helicase. *Science*, **275**, 377–380.
55. Dessinges,M.N., Lionnet,T., Xi,X.G., Bensimon,D. and Croquette,V. (2004) Single-molecule assay reveals strand switching and enhanced processivity of UvrD. *Proc. Natl Acad. Sci. USA*, **101**, 6439–6444.
56. Grossman,L., Caron,P.R., Mazur,S.J. and Oh,E.Y. (1988) Repair of DNA-containing pyrimidine dimers. *FASEB J.*, **2**, 2696–2701.
57. Van Houten,B., Gamper,H., Hearst,J.E. and Sancar,A. (1988) Analysis of sequential steps of nucleotide excision repair in *Escherichia coli* using synthetic substrates containing single psoralen adducts. *J. Biol. Chem.*, **263**, 16553–16560.
58. Caron,P.R., Kushner,S.R. and Grossman,L. (1985) Involvement of helicase II (uvrD gene product) and DNA polymerase I in excision mediated by the uvrABC protein complex. *Proc. Natl Acad. Sci. USA*, **82**, 4925–4929.
59. Husain,I., Van Houten,B., Thomas,D.C., Abdel-Monem,M. and Sancar,A. (1985) Effect of DNA polymerase I and DNA helicase II on the turnover rate of UvrABC excision nuclease. *Proc. Natl Acad. Sci. USA*, **82**, 6774–6778.
60. Hall,M.C., Jordan,J.R. and Matson,S.W. (1998) Evidence for a physical interaction between the *Escherichia coli* methyl-directed mismatch repair proteins MutL and UvrD. *EMBO J.*, **17**, 1535–1541.
61. Kosinski,J., Steindorf,I., Bujnicki,J.M., Giron-Monzon,L. and Friedhoff,P. (2005) Analysis of the quaternary structure of the MutL C-terminal domain. *J. Mol. Biol.*, **351**, 895–909.
62. Fischer,C.J., Maluf,N.K. and Lohman,T.M. (2004) Mechanism of ATP-dependent translocation of *E. coli* UvrD monomers along single-stranded DNA. *J. Mol. Biol.*, **344**, 1287–1309.
63. Sancar,A. and Hearst,J.E. (1993) Molecular matchmakers. *Science*, **259**, 1415–1420.
64. Robertson,A.B., Pattishall,S.R., Gibbons,E.A. and Matson,S.W. (2006) MutL-catalyzed ATP hydrolysis is required at a post-UvrD loading step in methyl-directed mismatch repair. *J. Biol. Chem.* In press.
65. Runyon,G.T. and Lohman,T.M. (1993) Kinetics of *Escherichia coli* helicase II-catalyzed unwinding of fully duplex and nicked circular DNA. *Biochemistry*, **32**, 4128–4138.
66. Runyon,G.T., Bear,D.G. and Lohman,T.M. (1990) *Escherichia coli* helicase II (UvrD) protein initiates DNA unwinding at nicks and blunt ends. *Proc. Natl Acad. Sci. USA*, **87**, 6383–6387.
67. Runyon,G.T. and Lohman,T.M. (1989) *Escherichia coli* helicase II (uvrD) protein can completely unwind fully duplex linear and nicked circular DNA. *J. Biol. Chem.*, **264**, 17502–17512.
68. Hall,M.C., Shcherbakova,P.V., Fortune,J.M., Borchers,C.H., Dial,J.M., Tomer,K.B. and Kunkel,T.A. (2003) DNA binding by yeast Mlh1 and Pms1: implications for DNA mismatch repair. *Nucleic Acids Res.*, **31**, 2025–2034.