Evidence for a physical interaction between the *Escherichia coli* methyl-directed mismatch repair proteins MutL and UvrD

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UvrD (DNA helicase II) is an essential component of two major DNA repair pathways in *Escherichia coli*: methyl-directed mismatch repair and UvrABC-mediated nucleotide excision repair. In addition, it has an undefined role in the RecF recombination pathway and possibly in replication. In an effort to better understand the role of UvrD in these various aspects of DNA metabolism, a yeast two-hybrid screen was used to search for interacting protein partners. Screening of an *E.coli* genomic library revealed a potential interaction between UvrD and MutL, a component of the methyl-directed mismatch repair pathway. The interaction was confirmed by affinity chromatography using purified proteins. Deletion analysis demonstrated that the C-terminal 218 amino acids (residues 398–615) of MutL were sufficient to produce the two-hybrid interaction with UvrD. On the other hand, both the N- and C-termini of UvrD were required for interaction with MutL. The implications of this interaction for the mismatch repair mechanism are discussed.

Keywords: mismatch repair/MutL/protein–protein interaction/two-hybrid system/UvrD

Introduction

The *Escherichia coli* methyl-directed mismatch repair system functions primarily to correct replication errors and is also involved in preventing recombination between divergent DNA sequences (for a recent review see Modrich and Lahue, 1996). Inactivation of this pathway results in elevated spontaneous mutation frequencies (Nevers and Spatz, 1975; Glickman and Radman, 1980). The mismatch repair pathway can be reconstituted *in vitro* with the activities of eight polypeptides (Lahue *et al.*, 1989). MutS, MutL and MutH are involved in initiation of repair including mismatch recognition and generation of a nick at a nearby GATC sequence (Su and Modrich, 1986; Su *et al.*, 1988; Grilley *et al.*, 1989; Au *et al.*, 1992). The hemimethylated state of GATC sequences immediately following replication serves as a signal to direct repair to the nascent strand of the DNA duplex (Lu *et al.*, 1983; Pukkila *et al.*, 1983). DNA helicase II and one of several exonucleases are required to excise the error-containing DNA strand beginning at the nicked GATC site (Cooper *et al.*, 1993; Grilley *et al.*, 1993). Restoration of the

correct DNA sequence by repair synthesis involves DNA polymerase III holoenzyme and SSB, and the final nick is sealed by DNA ligase (Lahue *et al.*, 1989).

Mismatch repair systems also exist in eukaryotic organisms (for recent reviews see Kunkel, 1995; Kolodner, 1996; Modrich and Lahue, 1996). Homologs of the bacterial MutS and MutL proteins have been identified in several eukaryotic organisms, including yeast and humans. Although the *in vivo* signal for strand discrimination and the mechanism of excision are unknown in eukaryotes, mismatch recognition involving the MutS and MutL homologs appears to be very similar to the bacterial system. In humans, defects in mismatch repair result in genomic instability that can lead to certain types of cancer, especially hereditary colon cancer (Karran and Bignami, 1994; Eshleman and Markowitz, 1995; Jiricny, 1996; Umar and Kunkel, 1996).

In addition to its role in removing mismatched bases, the product of the *E.coli* *uvrD* gene (DNA helicase II) performs a similar excision function for DNA lesions repaired by the UvrABC-mediated nucleotide excision repair system (Caron *et al.*, 1985; Husain *et al.*, 1985). Site-directed mutagenesis studies have demonstrated that function in both of these pathways requires the ATP hydrolysis-dependent helicase activity of UvrD (George *et al.*, 1994; Brosh and Matson, 1995, 1997). Although *E.coli* contains at least 11 DNA helicases (Matson, 1991), UvrD is the only helicase capable of functioning in the excision step of these two DNA repair pathways. It is probable that protein–protein interactions are the reason for this specificity and are responsible for directing the involvement of helicase II in DNA repair.

To identify interactions with other *E.coli* proteins that may be important for the cellular functions of UvrD, we used the yeast two-hybrid system with UvrD as bait. By screening an *E.coli* genomic library, MutL was identified as a potential interacting protein. MutL exists as a homodimer of 68 kDa polypeptides for which no enzymatic activity has been demonstrated. However, MutL binds to both single-stranded DNA (ssDNA) and double-stranded DNA (dsDNA) (Bende and Grafstrom, 1991) and displays affinity for MutS–mismatch complexes (Grilley *et al.*, 1989). It has previously been proposed to act as a bridge between components of the mismatch repair system (Modrich and Lahue, 1996). In this report, *in vitro* experiments confirm that UvrD and MutL make a direct physical interaction that may be required for function of the methyl-directed mismatch repair system.

Results

The two-hybrid screen

To identify *E.coli* proteins that potentially interact with DNA helicase II we constructed an *E.coli* genomic library

in the two-hybrid vector pGAD424 as described in Materials and methods. UvrD, expressed as a translational fusion with the Gal4 DNA binding domain in pGBT9, was used as bait. Potential interactions in the yeast two-hybrid reporter strain HF7c were initially selected on complete synthetic media lacking histidine. HF7c contains a *HIS3* gene under control of the *GAL1* promoter which is only expressed when a functional Gal4 protein is reconstituted by an interaction between the activation domain and DNA binding domain fusion proteins. Potential interactors in pGAD424-lib were recovered as described in Materials and methods, and the interaction was confirmed by retransformation with pGBT9-UvrD into HF7c and SFY526. SFY526 contains a *lacZ* reporter gene and its expression can be monitored with the color-producing substrates X-gal and ONPG. Library clones that maintained an interaction in HF7c and SFY526 which was dependent on the presence of UvrD were sequenced and subjected to a BLAST search of the *E.coli* genome database. One clone, designated I-24, was found to be 100% identical to a portion of the mismatch repair gene *mutL*. Sequence analysis revealed that the *mutL* portion of I-24 was in the same reading frame as the *GAL4* activation domain and began at base pair (bp) 879 of the published *mutL* coding sequence.

Full length UvrD and MutL proteins interact in the two-hybrid system

Since I-24 was missing the first 878 bp of the 1848 bp *mutL* gene, we were interested in examining the interaction between full length MutL and UvrD. To accomplish this, *mutL* was cloned from the *E.coli* genome into pGAD424 and pGBT9 as described in Materials and methods. The pGAD424-MutL and pGBT9-MutL clones were used to confirm the interaction with UvrD in HF7c and SFY526. Figure 1 shows that growth of HF7c on complete synthetic media lacking histidine was dependent on the presence of both *mutL* and *uvrD* in the two-hybrid vectors. The absence of *mutL*, *uvrD* or both resulted in basal levels of reporter gene expression and, therefore, no growth on media lacking histidine. Identical results were obtained in the *lacZ* reporter strain SFY526 with regard to β -galactosidase expression (data not shown). In addition, the interaction was observed regardless of whether MutL existed as a fusion with the Gal4 activation domain or the Gal4 DNA binding domain (Figures 1 and 2).

The N-terminal 397 amino acids of MutL are not required for the two-hybrid interaction with UvrD

A series of N- and C-terminal deletions was generated in the *mutL* and *uvrD* genes in an effort to localize the interaction domain to smaller regions of the two proteins. The *mutL* and *uvrD* deletions were constructed in pGAD424-MutL and pGAD424-UvrD, respectively, and assayed for their ability to activate *lacZ* expression in the presence of the appropriate interactor (either pGBT9-UvrD or pGBT9-MutL). β -galactosidase activity was measured using a spectrophotometric assay with the substrate ONPG, and the results are expressed as Miller units (Figure 2). Deletion of amino acids 1–100 (*uvrD* Δ 100N) or 681–720 (*uvrD* Δ 40C) from UvrD eliminated the two-hybrid interaction with MutL. These results indicated that, in the context of the yeast two-hybrid system, both the N- and

Fig. 1. MutL and UvrD interact in the yeast two-hybrid system. Yeast HF7c cells containing various constructs of pGBT9 and pGAD424 were grown at 30°C on complete synthetic media lacking tryptophan, leucine and histidine, and supplemented with 1 mM 3-AT. Each quadrant on both plates contains cells streaked from a single transformant that was colony-purified. Labels for both panels represent the fusion proteins present in HF7c in the order: DNA binding domain/transcriptional activation domain. (–) represents the absence of MutL or UvrD from the fusion construct.

C-termini of UvrD were essential for the interaction with MutL.

The N-terminal 293 amino acids of MutL were dispensable for the interaction with UvrD since I-24 (*mutL* Δ 293N) was isolated from the *E.coli* genomic library by virtue of its interaction with UvrD. Further deletions from the N-terminus of MutL (*mutL* Δ 344N and *mutL* Δ 397N) did not disrupt the interaction with UvrD. However, when the N-terminal 438 amino acids were removed (*mutL* Δ 438N), the interaction was lost. Also, deletion of 59 amino acids from the C-terminus of MutL (*mutL* Δ 59C) eliminated the interaction with UvrD. Therefore, critical residues for the interaction with UvrD are located between amino acids 397 and 438, and after amino acid 559 of MutL. Other critical residues may exist between amino acids 438 and 559, but this was beyond the scope of the deletion analysis performed here.

Purified MutL is specifically retained on a UvrD affinity column

MutL and UvrD were over-expressed and purified as described in Materials and methods. Affinity chromatography was used to demonstrate a physical interaction between the two purified proteins. A UvrD affinity column was generated using activated Affi-gel 10 resin. Quantitation of the coupling efficiency for this column revealed that ~3.1 mg of UvrD was coupled to a resin volume of 400 μ l. Fifty μ g of purified MutL or BSA were loaded onto the column and eluted with a series of salt washes as described in Materials and methods.

Fig. 2. Deletion analysis of the MutL–UvrD interaction in the yeast two-hybrid system. β -galactosidase activity was measured in yeast SFY526 cells using ONPG as described in Materials and methods. (A) All *mutL* deletions were constructed in pGAD424 and were tested for an interaction in the presence of pGBT9-UvrD. (B) All *uvrD* deletions were constructed in pGAD424 and were tested for an interaction in the presence of pGBT9-MutL. (+) indicates the presence of an interaction and (–) indicates the absence of an interaction. All interactions displayed at least a 50-fold increase in β -galactosidase activity (Miller units) compared with controls in the absence of *uvrD* and *mutL*. All results represent the average of at least three experiments using independent transformants.

As shown in Figure 3A, a large fraction of the applied MutL bound the UvrD affinity column and was eluted from the column with 250 mM and 1 M NaCl washes. A control protein (BSA) failed to bind the UvrD column and was found exclusively in the flow-through and 50 mM NaCl wash fractions (Figure 3B). In addition, a control affinity column was constructed by coupling ~3.4 mg of lysozyme to 400 μ l of Affi-gel 10 resin. Fifty μ g of MutL were loaded onto the control column, which was treated in the same manner as the UvrD column. Nearly all of the loaded MutL was present in the flow-through and 50 mM NaCl wash fractions (data not shown), indicating that MutL was not retained on the UvrD affinity column by non-specific interactions with the resin or protein. These results support the conclusion that a specific physical interaction exists between UvrD and MutL.

MutL has no effect on the ssDNA-stimulated ATPase activity of UvrD

Previously, Modrich and colleagues have observed that the helicase activity of UvrD is greatly stimulated in the presence of MutL (M.Yamaguchi and P.Modrich of Duke University, personal communication). We confirmed the MutL stimulation of UvrD-catalyzed unwinding using standard helicase assays with partial duplex and blunt duplex DNA substrates. There are several possible explanations for the stimulation of UvrD helicase activity. To examine one of these, we measured the effect of MutL on the ssDNA-stimulated ATPase activity of UvrD. The turnover rate (k_{cat}) for ATP hydrolysis catalyzed by UvrD was measured in the presence and absence of MutL (Table I). The k_{cat} values for UvrD-catalyzed ATPase activity were independent of MutL concentration. Results were

similar using M13mp7 ssDNA circles and oligonucleotide (dT)₁₆ as the ssDNA effectors of the ATPase reaction. Thus, MutL did not stimulate UvrD-catalyzed DNA unwinding by enhancing its rate of ATP hydrolysis.

Discussion

Escherichia coli DNA helicase II plays a specific role in the methyl-directed mismatch repair and nucleotide excision repair pathways (Caron *et al.*, 1985; Husain *et al.*, 1985; Lahue *et al.*, 1989), a less defined role in recombination (Horii and Clark, 1973; Arthur and Lloyd, 1980; Feinstein and Low, 1986; Schellhorn and Low, 1991; Mendonca *et al.*, 1993, 1995; Morel *et al.*, 1993) and possibly replication (Taucher-Scholz and Hoffman-Berling, 1983; Washburn and Kushner, 1991; George *et al.*, 1994; Brosh and Matson, 1995). We used the yeast two-hybrid system to search for interacting protein partners that might direct the involvement of helicase II in one or more of these processes. The two-hybrid screen revealed an interaction between helicase II and MutL, an essential component for methyl-directed mismatch repair and, according to a recent report, a factor in transcription-coupled nucleotide excision repair (Mellon and Champe, 1996).

Deletion analysis demonstrated that the C-terminal 218 amino acids of MutL were sufficient to maintain the two-hybrid interaction, suggesting that this region contains the interface for binding to UvrD. We were unable to localize the region of UvrD responsible for binding to MutL because removal of either the N- or C-terminus of UvrD eliminated the two-hybrid interaction. One explanation for this result is that the two-hybrid system was not truly

Fig. 3. MutL was specifically retained on a UvrD affinity column. Fifty μ g MutL (A) or BSA (B) were loaded onto a 400 μ l Affi-gel 10 column containing 3.1 mg covalently coupled purified UvrD as described in Materials and methods. In both (A) and (B): lanes 1–3, flow-through (FT) and washes; lanes 4–6, 250 mM NaCl elution; lanes 7 and 8, 1 M NaCl elution. Each lane represents 36 μ l of the corresponding fraction. All fractions were 400 μ l except the flow-through fraction, which was 1 ml. Gels were stained with Coomassie Brilliant Blue. Molecular weight markers (Bio-Rad) were: rabbit muscle phosphorylase b (97.4 kDa), BSA (66.2 kDa), hen egg white ovalbumin (45.0 kDa), bovine carbonic anhydrase (31.0 kDa) and soybean trypsin inhibitor (21.5 kDa).

reflective of the lack of an interaction in the case of one or both of these deletions due to lack of expression, protein degradation, insolubility or misfolding. However, both the UvrD Δ 100N and UvrD Δ 40C truncation mutants were expressed as stable, soluble proteins in the yeast strains (data not shown). It is possible that both termini of UvrD contain residues involved in the interaction with MutL. The interaction domain may be bipartite, or the two termini may actually be juxtaposed in the three-dimensional structure of UvrD. Future studies aimed at generating point mutants that exhibit defective interactions with MutL should aid identification of the interaction domain in UvrD.

The mismatch repair system displays bidirectional capability (Cooper *et al.*, 1993; Grilley *et al.*, 1993). Correction of a mismatched base can be directed by a hemimethylated GATC sequence on either the 5' or 3' side of the error. Helicase II participates in the excision of the DNA biosynthetic error by unwinding from the nicked GATC site to a location past the error. Interestingly, helicase II preferentially unwinds from the nick toward the mismatch, regardless of whether the nicked GATC sequence is on the 5' or 3' side (Grilley *et al.*, 1993).

Table I. The effect of MutL on the k_{cat} for ATP hydrolysis catalyzed by UvrD^a

[MutL] (nM)	ssDNA effector	
	M13mp7	oligo (dT) ₁₆
0	139 $\pm$ 6 s ⁻¹	83 $\pm$ 20 s ⁻¹
6	132 $\pm$ 14 s ⁻¹	81 $\pm$ 14 s ⁻¹
30	134 $\pm$ 4 s ⁻¹	65 $\pm$ 14 s ⁻¹
150	152 $\pm$ 30 s ⁻¹	70 $\pm$ 16 s ⁻¹

^aThe concentration of UvrD in each reaction was 4 nM.

This occurs despite the fact that helicase II alone catalyzes an unwinding reaction in both directions from a nicked DNA molecule *in vitro* (Runyon *et al.*, 1990). Because UvrD unwinds exclusively in a 3' to 5' direction with respect to the bound DNA strand (Matson, 1986; Georgi-Geisberger and Hoffmann-Berling, 1990), bidirectional unwinding from a nick necessitates the ability of UvrD to bind to both strands. These observations are consistent with the presence of a signal within the mismatch repair system that is used to orient helicase II, perhaps by loading it onto the appropriate DNA strand at the nicked GATC site, so that it unwinds in the proper direction for mismatch excision. It is possible that the interaction between MutL and UvrD serves this purpose.

Recently, it was shown that MutS translocates bidirectionally from a mismatch site in an ATP hydrolysis-dependent fashion, creating a looped DNA structure that usually contains the mismatch (Allen *et al.*, 1997). MutL stimulates the rate of this process and both proteins are found as a complex at the base of the loop structure. It is reasonable to think that the arrival of the MutS–MutL complex at a GATC site activates the MutH-associated endonuclease and allows initiation of unwinding by UvrD from the resulting nick. MutL may be required to load UvrD onto the nicked DNA to overcome occlusion of the nick by the initiation complex.

In addition to the aforementioned observations regarding the DNA unwinding activity of UvrD in the mismatch repair system, the Modrich laboratory has shown that the presence of MutL stimulates the *in vitro* helicase activity of UvrD (M. Yamaguchi and P. Modrich of Duke University, personal communication) and we have subsequently confirmed this result. Multiple mechanisms for MutL-stimulated helicase activity are possible. MutL did not stimulate UvrD ATPase activity using ssDNA effector molecules, even at a high molar concentration. Thus, enhancement of ATPase activity, which powers the unwinding reaction, is not a likely explanation. As mentioned above, it is possible that MutL facilitates the loading of UvrD onto a DNA molecule. This could explain the *in vitro* observations of Modrich and colleagues (Grilley *et al.*, 1993) that UvrD preferentially unwinds toward the error in the context of the mismatch repair system. Alternatively, MutL could act as a processivity factor, or clamp, serving to maintain a productive interaction between UvrD and the DNA substrate during an unwinding reaction. These possibilities will be the subject of future studies.

The involvement of MutL in transcription-coupled nucleotide excision repair, a subset of the excision repair reactions of which UvrD is an essential component,

raises an interesting question regarding the MutL–UvrD interaction in this pathway. Although MutL is not required to reconstitute the nucleotide excision repair system *in vitro* (Hoeijmakers, 1993; Sancar, 1996), *mutL* mutants are slightly sensitive to UV light and are deficient in repair of UV-induced DNA lesions in transcriptionally active genes (Mellon and Champe, 1996). We are currently exploring the functional importance of the MutL–UvrD interaction for both methyl-directed mismatch repair and UvrABC-mediated nucleotide excision repair.

The identification of an interaction between MutL and UvrD may have important consequences for understanding mismatch repair in eukaryotic systems. Defects in eukaryotic mismatch repair result in destabilization of short repetitive sequences and have been linked to various cancers, most notably hereditary colon cancer (Karran and Bignami, 1994; Eshleman and Markowitz, 1995; Jiricny, 1996; Umar and Kunkel, 1996). The error recognition steps of eukaryotic mismatch repair are somewhat more complex than in prokaryotes, but nevertheless occur by a similar mechanism involving homologs of the bacterial MutS and MutL proteins (Kolodner, 1996; Modrich and Lahue, 1996). In fact, a number of eukaryotic mismatch repair genes were identified based on their homology to bacterial *mutS* and *mutL* (reviewed in Fishel and Kolodner, 1995). Despite the striking similarities between the initiation steps of prokaryotic and eukaryotic mismatch repair, very little is known about the subsequent steps in the eukaryotic pathway. For instance, an *in vivo* strand discrimination signal has not been discovered, and the mechanisms and proteins involved in excision and repair synthesis remain enigmatic.

Of particular significance is the fact that no helicase has yet been identified as a participant in a eukaryotic mismatch repair system. Given the conservation of function of MutL and its eukaryotic homologs, and the interaction between MutL and UvrD in *E. coli* mismatch repair, it is possible that a similar interaction exists in eukaryotes. As of yet, such an interaction has evaded detection and it should be noted that sequence alignments of MutL with its eukaryotic homologs (Kramer *et al.*, 1989; Prolla *et al.*, 1994) reveal a high degree of similarity near the N-terminus and little similarity near the C-terminus, which we have shown contains the UvrD interaction domain. It is possible that eukaryotic mismatch repair does not require a helicase; one or more nucleases may be solely responsible for the excision step, for example. Alternatively, the DNA polymerase involved in the repair synthesis step might be capable of strand displacement synthesis in the context of the mismatch repair pathway. If the overall mechanism of mismatch correction is conserved from *E. coli* to eukaryotes, then functional redundancy among multiple helicases may have prevented discovery of their involvement in mismatch repair by conventional screening procedures. Whatever the case, the discovery of a physical interaction between UvrD and MutL in *E. coli* may lead to advances in our understanding of mismatch repair in eukaryotic systems.

Materials and methods

Strains, enzymes, DNA and nucleotides

Escherichia coli JS4 was from Bio-Rad. *Escherichia coli* HB101 was from New England Biolabs (NEB). *Escherichia coli* BL21 was from

Novagen. *Escherichia coli* K-12 was a gift from E. Raleigh (NEB). GE1752Δ*uvrD* was constructed previously in this laboratory (Mendonça *et al.*, 1993). Yeast HF7c and SFY526, and plasmids pGAD424 and pGBT9 were from the Matchmaker two-hybrid system (Clontech). pCYB2 and other components of the Impact I protein purification system were from NEB. pET9d-UvrD was constructed previously in this laboratory (George *et al.*, 1994). pLitmus was from NEB. M13mp7 ssDNA was prepared as previously described (Lechner and Richardson, 1983). Oligonucleotide (dT)₁₆ was from The Midland Certified Reagents Company. Radiolabeled ATP was from Amersham Life Sciences, Inc. and unlabeled nucleotides were from US Biochemicals Corporation. All DNA modifying enzymes used for cloning and PCR were from NEB with the exception of T4 DNA ligase, which was from Boehringer Mannheim. Bovine serum albumin (BSA) was from NEB and lysozyme was from Sigma.

Library construction

Escherichia coli K-12 genomic DNA was prepared on a CsCl/EtBr gradient as previously described (Ausubel *et al.*, 1995) and partially digested with 1 unit *Sau3A* per 6 μg DNA for 20 min at 37°C. Resulting DNA fragments were separated by size on a gradient of 10–40% sucrose in STE buffer (100 mM NaCl, 10 mM Tris–HCl pH 7.5, 1 mM EDTA). Centrifugation was performed at 85 500 *g* in a Beckman L8–70M ultracentrifuge for 24 h. Fractions containing DNA fragments in the 4–7 kbp size range were pooled and DNA was recovered by ethanol precipitation. The DNA fragments were subsequently ligated into the *Bam*HI site of pGAD424 at an insert:vector molar ratio of 5:1, and transformed by electroporation into *E. coli* JS4. Approximately 1 × 10⁶ transformant colonies were suspended in LB media and the cells recovered by centrifugation. Plasmid DNA (pGAD424-lib) was purified as described (Ausubel *et al.*, 1995), using a CsCl/EtBr gradient. The fraction of recombinant clones was determined to be ~62%. This was sufficient to ensure that all possible clones were present in the library, in the correct orientation, with a probability of 99%, based on the assumption that *Sau3A* restriction sites occur, on average, every 256 bp in the *E. coli* genome.

Cloning *uvrD* and *mutL*

Vent DNA polymerase was used to amplify the *uvrD* gene by PCR using pET9d-UvrD as target. Amplified *uvrD* was cloned into the *Sma*I site of pGAD424 and pGBT9 to create in-frame translational fusions with the Gal4 transcriptional activation domain and DNA binding domain, respectively. These constructs were designated pGAD424-UvrD and pGBT9-UvrD. In similar fashion, the *mutL* gene was amplified by PCR from *E. coli* K-12 genomic DNA and directionally cloned into the *Eco*RI and *Bam*HI sites of pGAD424 and pGBT9 to form the appropriate translational fusions. These constructs were designated pGAD424-MutL and pGBT9-MutL. The *mutL* PCR primers also allowed cloning of *mutL* into the *Nde*I and *Sma*I sites of pCYB2 as a translational fusion with the intein and chitin binding proteins for over-expression and purification using the Impact I system (NEB).

Deletion construction

Deletions of the *uvrD* and *mutL* genes in pGAD424 were generated using the cloning strategy outlined above, and either convenient restriction enzyme sites or PCR primers designed to amplify the appropriate region. Specific PCR primers were designed to create *mutL*Δ344N and *mutL*Δ397N. The following restriction enzyme sites were used to generate other deletions from the PCR-amplified genes described in the preceding paragraph: *Fsp*I for *uvrD*Δ100N, *Ssp*I for *mutL*Δ59C and *Rsa*I for *mutL*Δ438N. The *uvrD*Δ40C deletion was created by digestion of pGAD424-UvrD with *Bsi*WI and *Bgl*II. The 5'-overhang ends of the vector were filled in using DNA polymerase I (large fragment) and covalently closed plasmid DNA was restored by blunt ligation. The creation of in-frame translational fusions was confirmed by sequence analysis.

Detection of reporter gene expression

pGBT9-UvrD and pGAD424-lib were cotransformed into yeast HF7c cells and plated on complete synthetic media lacking leucine, tryptophan and histidine, and supplemented with 1 mM 3-amino-1,2,4-triazole (3-AT). 3-AT was required to suppress a low level of *HIS3* activation by the pGBT9-UvrD DNA binding domain fusion. Transformation efficiencies were monitored by plating small portions of transformations on complete synthetic media lacking only leucine and tryptophan. Transformants capable of growth in the absence of histidine were colony-purified and DNA was extracted as described by the supplier (Clontech).

The pGAD424-lib plasmid was recovered in each case by transformation into *E.coli* HB101 and selecting for growth on minimal media lacking leucine as described by the supplier. Recovered pGAD424-lib plasmids were retransformed with pGBT9-UvrD into yeast HF7c and SFY526 cells. Confirmation of an interaction was performed by monitoring growth of HF7c on media lacking histidine and the appearance of blue color in SFY526 in the presence of X-Gal. A spectrophotometric assay for β -galactosidase activity, using the substrate o-nitrophenyl β -D-galactopyranoside (ONPG), allowed quantification of experimental and control interactions (Miller, 1972), and was performed as described (Clontech).

Purification of UvrD and MutL

UvrD was over-expressed and purified as previously described (Runyon *et al.*, 1993; Hall and Matson, 1997). To over-express the MutL-intein fusion protein, either *E.coli* BL21 or GE1752 Δ uvrD containing pCYB2-MutL were grown in LB media to an optical density (600 nm) of $\sim$ 1.0 at 37°C. After addition of isopropyl β -D-thiogalactopyranoside to 0.5 mM, cells were incubated for an additional 5 h at 30°C to allow protein expression.

MutL was initially purified according to the supplied protocol (Impact I system, NEB). The chitin-binding portion of the MutL-intein fusion allowed retention of this protein on a chitin column. DTT-induced self-cleavage by the intein resulted in elution of highly enriched MutL. Subsequently, a Bio-Rex 70 column was used to separate MutL from a single prominent contaminating protein of $\sim$ 70 kDa. Pooled fractions from the Impact I chitin column were dialyzed against equilibration buffer (30 mM KPO₄ pH 7.4, 50 mM KCl, 1 mM 2-mercaptoethanol, 10% glycerol and 0.1 mM EDTA) and loaded onto the Bio-Rex 70 column at 0.5 ml/min. Protein was eluted from the column with a linear KCl gradient (50–350 mM) and fractions were analyzed by SDS-PAGE. Fractions eluting at $>$ 250 mM KCl were highly enriched for MutL in comparison with the 70 kDa contaminant and were pooled and dialyzed against MutL storage buffer (50 mM KPO₄ pH 7.4, 50 mM KCl, 1 mM 2-mercaptoethanol, 50% glycerol and 0.1 mM EDTA). Subjecting the initial cleared cell lysate to a 0.2% polymin P precipitation and 50% ammonium sulfate precipitation did not eliminate the contaminating protein. Due to the chemistry of the intein cleavage reaction in the Impact I system, the purified MutL contained a single extra amino acid (glycine) at the C-terminus.

Affinity chromatography

3.75 mg of purified UvrD were dialyzed against coupling buffer (25 mM HEPES pH 7.0, 10% glycerol and 200 mM NaCl). Approximately 400 μ l Affi-gel 10 resin (Bio-Rad) were added as described by the supplier. The mixture was incubated at 4°C for 4 h to allow covalent coupling of UvrD to the activated resin and quenched for 1 h with $\sim$ 15 mM ethanolamine pH 8.0. 3.6 mg lysozyme were dissolved in coupling buffer and treated exactly as described above. Coupling efficiencies (82% for UvrD and 94% for lysozyme) were determined by comparing concentrations of the pre-coupling protein sample with concentrations of the protein collected by extensive washing of the coupled resin.

To evaluate binding to the UvrD and lysozyme affinity resins, 50 μ g protein in a volume of 1 ml affinity buffer (25 mM Tris pH 7.5, 10% glycerol and 2.5 mM 2-mercaptoethanol) containing 50 mM NaCl was loaded onto the affinity column at a flow rate of 4–5 ml/h. The column was then washed with three 400 μ l aliquots of affinity buffer plus 50 mM NaCl, and eluted with four 400 μ l aliquots each of affinity buffer plus 250 mM NaCl and 1 M NaCl. Each wash was collected independently and analyzed by electrophoresis on a 10% polyacrylamide gel in the presence of SDS.

ATPase assays

Standard conditions for ATPase reactions were 25 mM Tris-HCl pH 7.5, 20 mM NaCl, 3 mM MgCl₂ and 5 mM 2-mercaptoethanol. ATPase reactions (30 μ l) containing 1 mM [α -³²P]ATP and 150 μ M M13mp7 ssDNA (nucleotide phosphate) were initiated with UvrD, MutL or a mixture of both proteins, at the indicated concentrations, and incubated at 37°C. Samples (5 μ l) were removed at 1 or 2 min intervals and evaluated by thin-layer chromatography as previously described (Matson and Richardson, 1983). Results were analyzed using phosphor storage technology and ImageQuant software (Molecular Dynamics). Background levels were always $<$ 2% and were subtracted from the experimental data.

Acknowledgements

We thank Drs Thomas Kunkel and Thomas Petes for critical reading of the manuscript and Susan Whitfield for preparation of the figures. We also thank Dr Paul Modrich for communication of results prior to publication. This work was supported by National Institutes of Health Grant GM-33476 to S.W.M.

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Received November 28, 1997; revised and accepted January 8, 1997

Note added in proof

Manuscripts (Yamaguchi,M., Dao,V. and Modrich,P. 'MutS and MutL activate DNA helicase II in a mismatch-dependent manner' and Dao,V. and Modrich,P. 'Mismatch, MutS, MutL and helicase II-dependent unwinding from the single-stranded break of an incised heteroduplex') describing the biochemical interaction between helicase II and MutL are in press in *J. Biol. Chem.*