Heteroduplex DNA and ATP induced conformational changes of a MutS mismatch repair protein from *Thermus aquaticus*

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ATP hydrolysis by MutS homologues is required for the function of these proteins in mismatch repair. However, the function of ATP hydrolysis in the repair reaction is not very clear. We have examined the role of ATP hydrolysis in oligomerization of *Thermus aquaticus* (Taq) MutS protein in solution. Analytical gel filtration and cross-linking of MutS protein with disuccinimidyl suberate suggest that TaqMutS is a dimer in the presence of ATP. ATP binding and hydrolysis by TaqMutS reduces the heteroduplex-DNA binding by the protein. Using limited proteolysis we detected extensive conformational changes of the TaqMutS protein in the presence of ATP and heteroduplex

DNA. Heteroduplex-DNA binding is necessary for the observed conformational changes since F39A mutant protein defective in DNA binding does not display ATP-induced conformational changes. The implications of the observed conformational changes in the MutS protein are discussed with respect to two different models proposed for the role of ATP hydrolysis by MutS in DNA mismatch repair.

Key words: cross-linking, DNA binding, nucleotide cofactors, proteolysis, TaqMutS.

INTRODUCTION

Mismatches in DNA can arise through replication error, physical damage to bases (e.g. deamination of 5-methylcytosine) and by the formation of heteroduplex DNA during genetic recombination between similar but not identical sequences (reviewed in [1]). In bacteria, these mismatches become fixed in the genome as mutations if left unrepaired. The most thoroughly understood mismatch-repair system is methyl-directed mismatch repair in *Escherichia coli* [1–6]. Three proteins MutS, MutL and MutH play a crucial role in this pathway. A model for the initiation of mismatch repair by MutS, MutL and MutH, immediately after passage of a DNA-replication fork, has been proposed based on studies both *in vivo* and *in vitro* [2]. In this model, a dimer of MutS first recognizes and binds to mismatches. In a reaction that requires ATP, a dimer of MutL binds to MutS dimer and then activates the methylation-sensitive endonuclease MutH. Activation of MutH results in cleavage of the unmethylated DNA strand at hemi-methylated dGATC sites that are transiently present after replication-fork passage, providing an entry point for excision and replication proteins to remove the mismatch and repair the resulting gap using the parental DNA strand as template.

Bacterial MutS proteins have an associated ATPase activity that is required for function of the protein in mismatch repair. In mutator assays, inactivation of the nucleotide-binding site results in a dominant negative phenotype [5]. The nucleotide-binding sequence is conserved in eukaryotic MutS homologues, and studies with mutants in this region have implicated the role of the nucleotide-binding centre of yeast MSH2, human MSH2 and human MSH6 in mismatch repair [7–11]. Because MutS homologues bind with high specificity to heteroduplex DNA in the absence of ATP, ATP hydrolysis by these proteins presumably functions at steps subsequent to mismatch recognition.

Two different models have been proposed for the role of

nucleotide binding and hydrolysis by MutS activities. In the translocation model, MutS uses energy derived from ATP hydrolysis to translocate along the DNA contour, a mechanism that would permit coupling of mismatch recognition to recognition of a secondary site that determines the strand specificity of the reaction [12–15]. In the molecular-switch model, the process of ATP binding and hydrolysis serves to switch the protein between two states, one of which is active in mismatch binding and one which is not, somewhat analogous to the signalling by the G-protein family using GTP hydrolysis [15].

In this work we studied the conformational changes in MutS protein induced by DNA binding in the presence of nucleotide cofactors. We employed limited proteolysis to monitor the conformational changes that occur in the MutS–heteroduplex-DNA complex. These conformational changes of the MutS protein are discussed in the context of the two models proposed for the role of ATP hydrolysis in DNA mismatch repair.

MATERIALS AND METHODS

Reagents

Wild-type *Thermus aquaticus* MutS (TaqMutS) and F39A mutant MutS proteins were purified as described previously [16,17]. ATP, ADP and AMP were from Sigma. ATP analogues adenosine 5'-[γ -thio]triphosphate (ATP[S]) and adenosine 5'-(β , γ -imino)triphosphate (AMPPNP) were from Fluka Chemicals. Chemical cross-linker disuccinimidyl suberate (DSS) was obtained from Pierce. Sequencing-grade chymotrypsin and other proteases were from Boehringer Mannheim.

DNA binding

DNA filter-binding assays were carried out as described previously [17] with the following modifications. MutS protein (20 pmol) was incubated with 5 pmol of ³²P-labelled 37-bp

Abbreviations used: TaqMutS, *Thermus aquaticus* MutS; ATP[S], adenosine 5'-[γ -thio]triphosphate; AMPPNP, adenosine 5'-(β , γ -imino)triphosphate; DSS, disuccinimidyl suberate.

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heteroduplex DNA [18,19] in 50 μ l of binding buffer (20 mM Hepes, pH 7.8, 5 mM $MgCl_2$, 0.1 mM EDTA and 10 % glycerol). Reactions were incubated at 60 °C for 15 min. ATP or other nucleotide analogues were then added to the reaction final concentration of 1 mM and further incubated at 60 °C for 10 min. Following incubation, samples were analysed by filter binding to nitrocellulose membrane (Schleicher and Schull, Keene, NH, U.S.A.) as described by Biswas and Hsieh [17]. Reactions were also carried out by preincubating MutS with various nucleotide cofactors at 60 °C for 15 min before addition of ^{32}P -labelled heteroduplex DNA. The sequences for 37-bp homoduplex (A:T) and heteroduplex (Δ 1) DNA were: top strand for both A:T and Δ 1, 5'-ATACCGTCGACGCTAGCGTGCG-GCTCGTCGTCGACCT-3'; bottom strand for A:T, 5'-AGG-TCGACGACGAGCCGCACGCTAGCGTCGACGGTAT-3'; and bottom strand for Δ 1, 5'-AGGTCGACGACGAGCCGCC-GCTAGCGTCGACGGTAT-3'.

Gel filtration

Gel filtration was carried out on a Superdex S-200 HR 10/30 column (Pharmacia). Approx. 100 μ g of MutS protein was incubated at 60 °C for 15 min in 100 μ l of buffer (20 mM Hepes, pH 7.8, 100 mM NaCl, 5 mM $MgCl_2$, 0.1 mM EDTA and 10 % glycerol). Reaction mixtures were cooled to 4 °C, applied to a Superdex S-200 column and eluted with the same buffer. For the analysis of MutS with ATP, 100 μ g of MutS was incubated in the above buffer with 1 mM ATP at 60 °C for 15 min, cooled to 4 °C and then applied to the column followed by elution with buffer containing 1 mM ATP. To find out the elution position of MutS, fractions were collected and MutS protein was detected in the fractions by the Bradford assay.

Chemical cross-linking

MutS protein (1 μ M) was incubated with the indicated nucleotide cofactors at 60 °C for 15 min in 20 μ l of buffer (20 mM Hepes, pH 7.8, 5 mM $MgCl_2$, 0.1 mM EDTA and 10 % glycerol). DSS was added at the indicated concentration and reactions were incubated at room temperature for 30 min and terminated by the addition of 1 μ l of Tris/HCl, pH 7.0, followed by an additional 15 min of incubation. Samples were analysed on by SDS/PAGE (6 % gel) after addition of 20 μ l of sample loading buffer and visualized by Coomassie Brilliant Blue staining.

Partial proteolysis of MutS

Partial chymotryptic digestion of MutS protein with and without cofactors was performed in the following way. MutS (80 pmol) in 20 μ l of binding buffer was incubated with or without 80 pmol of homoduplex or heteroduplex DNA as indicated. Reactions were incubated at 60 °C for 15 min in the presence or absence of a 1 mM concentration of the indicated nucleotide cofactors. Reaction were cooled down to room temperature and the indicated amount of sequencing-grade chymotrypsin (Boehringer Mannheim) was added and further incubated at 37 °C for 15 min. Reactions were terminated by addition of 20 μ l of 2 $\times$ SDS sample loading buffer and analysed by SDS/PAGE (8 % gel) followed by Coomassie Brilliant Blue staining.

RESULTS

Effect of ATP on the oligomerization of MutS protein in free solution

MutS proteins from prokaryotes are present as homodimers, whereas MutS from eukaryotes are generally present as hetero-

Figure 1 Gel filtration and cross-linking analysis of MutS in the presence of nucleotide cofactors

(A) Upper panel: MutS in binding buffer containing $MgCl_2$ was analysed on a Superdex S-200 gel-filtration column. Arrows indicate the elution position of standards (Kd = kDa). Lower panel: elution profile of MutS in the presence of 1 mM ATP as monitored by analysing the fractions by Bradford assay. Ve, exclusion volume; Vi, inclusion volume. (B) MutS was cross-linked with DSS in the absence (—NTP) or presence of 1 mM of the indicated nucleotide cofactors and analysed by SDS/PAGE (6 % gel) as described in the Materials and methods section. Concentrations of DSS were: 12.5 (lanes 3, 7, 11, 15), 25 (lanes 4, 8, 12, 16), 50 (lanes 5, 9, 13, 17) and 100 (lanes 6, 10, 14, 18) μ M. Intra-chain cross-linked monomeric and cross-linked dimeric species are indicated by arrows. Lanes 1 and 19, molecular-mass markers (Life Technologies). Lane 2, *Taq*MutS protein without any DSS cross-linker.

dimers in free solution ([12,19] and references therein). In order to study the effect of ATP hydrolysis on the oligomerization of *Taq*MutS, we employed two different techniques. In the first method, approx. 1 pmol of MutS protein was incubated in the presence of 5 mM ATP at 60 °C for 15 min in buffer containing $MgCl_2$ to allow ATP hydrolysis. An excess of ATP was present to ensure maximum hydrolysis by MutS. The reaction was then applied to analytical gel-filtration chromatography on a Superdex S-200 column and eluted in the presence of 1 mM ATP. As a control, MutS was incubated and eluted in the absence of ATP. The molecular mass of a *Taq*MutS monomer is 90.6 kDa, and in free solution it is a dimer that behaves anomalously and elutes at approx. 280 kDa in gel-filtration chromatography [17,19]. In our case, we observed that *Taq*MutS eluted as a 280 kDa protein in the presence or absence of ATP (Figure 1A), corresponding to a

Figure 2 Effect of nucleotide cofactors on DNA binding

(A) Filter-binding assays were performed using *Taq*MutS and 32 P-labelled 37-bp heteroduplex DNA as described in the Materials and methods section. Various nucleotide cofactors were also included in the reaction mixture together with the heteroduplex DNA as indicated. CONT, control reaction without any proteins; STD, standard binding reactions with MutS protein in the absence of any nucleotide cofactors. (B) Filter-binding assay of MutS with heteroduplex DNA in the presence of various amounts of ATP and AMPPNP. Reactions were done as in (A).

Stokes radius of 55.8 Å, as reported earlier [19]. This finding suggests that the oligomeric state of MutS protein is not altered upon ATP hydrolysis.

The oligomeric state of MutS protein was examined by chemical cross-linking using DSS, a non-specific cross-linker that targets primary amines. MutS (1 μ M) was first incubated in the presence of 1 mM ATP, 1 mM ADP or 1 mM ATP[S], or with no nucleotide cofactors, at 60 °C for 10 min in buffer containing $MgCl_2$ to allow ATP hydrolysis. MutS protein was then cross-linked with varying concentrations of DSS. At low DSS concentrations, several species were observed in the presence and absence of cofactors (Figure 1B). Among them, two species, representing monomers, migrated at 90 and 115 kDa. The latter species was probably due to intra-chain cross-linking of monomer species. In addition, higher-order complexes at or above 220 kDa migrating near the exclusion limit of SDS/PAGE (6% gel) were also observed. At higher concentrations of DSS, all the monomer species was converted almost entirely to the higher-order complex. The higher-order complex that migrates near the exclusion limit of the gel probably represents a cross-linked dimer species, as shown previously by MS analysis [19]. We obtained similar cross-linking patterns with and without different

nucleotide cofactors. Taken together, these results suggest that neither ATP binding nor hydrolysis change the oligomerization of MutS protein in free solution.

DNA binding in the presence of nucleotide cofactors

Previous studies using *E. coli* and eukaryotic MutS homologues indicated that mismatch substrate specificity of these proteins was abolished when binding reactions were performed in the presence of ATP. We tested whether *Taq*MutS displays similar properties by using a double filter-binding assay. *Taq*MutS was incubated at 60 °C with 32 P-labelled insertion/deletion heteroduplex DNA to allow MutS to bind to DNA. To the reaction mixture, 1 mM ATP, 1 mM ATP[S] or 1 mM AMPPNP was added and levels of mismatch binding were measured after incubation at 60 °C. As shown in Figure 2(A), in the presence of ATP, 68% binding was obtained compared with 86% binding in the absence of ATP (Figure 2A, STD). This is an approx. 20% reduction compared with the standard reaction without ATP. When poorly hydrolysable ATP[S] was included in the reaction, a greater reduction was observed (only 55% binding, a 36% reduction compared with the standard). However, when non-hydrolysable AMPPNP was present, it reduced the DNA binding to only 7.5%. In contrast, ADP did not alter the DNA-binding ability to a great extent; up to 81% DNA binding was obtained. We also carried out a binding reaction in which *Taq*MutS was initially preincubated at 60 °C in the presence or absence of ATP or its analogues, which was followed by the addition of 32 P-labelled heteroduplex DNA and further incubation at 60 °C to allow DNA binding. The levels of mismatch DNA binding were measured by filter binding as above. This was to verify the effect of ATP and its analogues on the existing MutS–heteroduplex-DNA complex. The effect of ATP and its analogues on DNA binding was largely similar to the earlier results (not shown). Our observations that ATP or its analogues inhibits MutS–DNA complex formation suggest that ATP binding and not ATP hydrolysis is sufficient for the MutS protein to dissociate from mismatches. This result is consistent with the previous findings with bacterial MutS and human MutS α [7,11,17,19–21]. We did not observe any significant reduction of DNA binding by *Taq*MutS in the presence of ADP (only 5% reduction, Figure 2A). This is also consistent with the earlier observation that ADP has very little effect on DNA binding by yeast or human MutS α [17,19]. Interestingly, we have observed a remarkable decrease of MutS binding to heteroduplex DNA in the presence of the non-hydrolysable ATP analogue AMPPNP (7.5% DNA binding compared with 87%). In an attempt to further investigate the inhibition by ATP and AMPPNP, we performed heteroduplex-DNA binding in the presence of various amounts of ATP or AMPPNP. As shown in Figure 2(B), inhibition was more pronounced with AMPPNP than with ATP and in both cases inhibition reached a plateau at 100 μ M. A further increase in concentration did not significantly influence heteroduplex binding. This result suggests that AMPPNP has a greater inhibitory effect on heteroduplex-DNA binding than ATP. This may be because AMPPNP, which is a non-hydrolysable ATP analogue, may irreversibly bind to the P-loop motif on the MutS protein and allosterically inhibit DNA binding, whereas ATP binds to MutS and after hydrolysis is released from the protein, allowing MutS to bind heteroduplex DNA.

Limited chymotryptic digestion of MutS protein

The translocation and molecular-switch models postulate different roles for ATP binding and hydrolysis in the function of

Figure 3 Limited proteolysis of *TagMutS* by chymotrypsin

MutS (80 pmol) was preincubated at 60 °C for 15 min with either ATP (1 mM) or ATP (1 mM) and heteroduplex DNA under binding conditions. Reactions were subjected to chymotrypsin digestion and analysed on an 8% SDS/PAGE gel as described in the Materials and methods section. Concentration of chymotrypsin are 0.1 μg (lanes 7, 13, 19), 0.2 μg (lanes 6, 12, 18), 0.5 μg (lanes 5, 11, 17), 1.0 μg (lanes 4, 10, 16), 2.0 μg (lanes 3, 9, 15) and 3.0 μg (lanes 2, 8, 14). Asterisks indicate major proteolytic fragments and M denotes broad-range molecular-mass markers (Bio-Rad).

MutS homologues. Since ATP binding or its hydrolysis did not change the oligomerization of MutS protein, this prompted us to investigate whether ATP binding or hydrolysis induces any conformational changes in the MutS protein, as proposed in the translocation model and molecular-switch model. Limited proteolysis has been demonstrated to be a simple useful tool for the detection of functional domains and cofactor-induced conformational changes in various proteins [22–26]. We used chymotrypsin, an endoprotease that specifically cleaves peptide bonds at the C-terminal ends of tyrosine, phenylalanine and tryptophan. It also cleaves after leucine, methionine, alanine, aspartic acid and glutamic acid residues, but at lower rates. There are over 50 major chymotryptic cleavage sites in *TagMutS* and selective hydrolysis of a subset of peptide bonds would constitute evidence for their exposure to solvent. MutS (80 pmol) was incubated in the presence or absence of 1 mM ATP and with or without 80 pmol of heteroduplex DNA in buffer containing $MgCl_2$ to allow ATP hydrolysis and DNA binding. The reaction mixture was then subjected to limited proteolysis with increasing amounts of chymotrypsin followed by SDS/PAGE analysis. Analysis of the kinetics of proteolysis reveals that not all sites are accessible to chymotryptic digestion as expected. However, there were at least five major peptide fragments generated along with 10–12 minor bands, as visualized by SDS/PAGE (Figure 3). The presence or absence of ATP in the reaction had no effect on the proteolytic pattern, suggesting that there was no significant conformational changes in the MutS protein in the presence of ATP that could be detected by chymotrypsin (Figure 3, lanes 2–7 and 8–13). In contrast, presence of both ATP and heteroduplex DNA in the reaction showed distinct changes in the kinetics of appearance as well as the pattern of proteolytic fragments (Figure 3, lanes 14–19). This suggests strongly that binding to heteroduplex DNA in the presence of ATP induces conformational changes in the MutS protein and that these conformational changes are not detected with ATP alone. This conformational change may be required for the observed differences in DNA binding in the presence or absence of ATP, as proposed in the molecular-switch model, or required for the

Figure 4 Effect of various nucleotide cofactors and DNA on proteolysis

MutS protein was incubated in the presence of 37-bp homoduplex or heteroduplex and nucleotide cofactors as indicated at 60 °C for 15 min to allow DNA binding. Reactions were subjected to chymotryptic digestion and analysed by SDS/PAGE (8% gel) as described in Figure 3. Arrows indicate the new proteolytic fragments obtained in the presence of ATP and heteroduplex DNA.

ATP-induced conformational changes in MutS that allows it to leave mismatches, as proposed in the translocation model.

Effects of DNA and cofactors on chymotryptic pattern of MutS

Since we have observed conformational changes in the MutS protein with heteroduplex DNA in the presence of ATP, we wanted to investigate further whether heteroduplex-DNA binding and ATP hydrolysis are indeed needed for the observed conformational changes. MutS was first incubated with or without homoduplex and heteroduplex DNA to allow DNA binding. ATP and its analogues were then added followed by limited proteolysis, as shown in Figure 4. We observed that homoduplex DNA alone or in the presence of ATP does not induce any significant changes in the chymotryptic pattern whereas heteroduplex DNA with ATP induces significant changes (Figure 4, lanes 4–6). However, the binding of heteroduplex DNA in the presence of AMP, ADP, ATP[S] and AMPPNP did not induce any conformational changes (Figure 4, lanes 7–10). This result suggests that ATP hydrolysis and not nucleotide binding alone with heteroduplex DNA is needed for conformational changes. Control experiments with homoduplex DNA in the presence of ADP, ATP[S] and AMPPNP were also performed (results not shown). We failed to observe any conformational changes, suggesting that DNA binding is necessary or that binding to homoduplex DNA is too transient to detect any conformational changes.

Effect of DNA and cofactors on chymotryptic digestion pattern of F39A MutS

The observation that heteroduplex-DNA binding is necessary for ATP-induced conformational changes in the MutS protein was verified further using F39A mutant protein, which retains all

Figure 5 Chymotryptic pattern of F39A mutant protein

F39A mutant MutS was preincubated with ATP and DNA as indicated, followed by chymotryptic digestion and analysis by SDS/PAGE as described in Figure 3.

the properties of wild-type MutS except that it is severely impaired in DNA binding [16]. Digestion of F39A by chymotrypsin under the same conditions generated identical proteolytic patterns compared with wild-type MutS, suggesting that there is no gross conformational changes due to mutation in F39A protein (Figure 5, lane 2). Limited proteolysis was carried out with F39A protein under the same conditions with or without heteroduplex DNA and in the presence or absence of ATP. As shown in Figure 5, no obvious change in the conformation was observed, as judged by the proteolytic pattern of F39A protein. This result strongly suggests that heteroduplex-DNA binding is indeed necessary for the observed ATP-induced conformational changes.

DISCUSSION

We have examined the effect of ATP and its analogues on the structural features of MutS protein. Gel filtration of MutS protein in the presence of ATP indicated that ATP hydrolysis does not affect the dimerization of MutS protein. This result was confirmed by chemical cross-linking studies with ATP, ADP and the poorly hydrolysable analogue ATP[S]. Cross-linking results also suggest that ATP binding or ADP binding by MutS protein as well as ATP hydrolysis did not change dimerization of MutS in free solution. It is generally believed that ATP is required for the loading of MutL on MutS bound to DNA [21]. Since ATP did not change the oligomeric nature of MutS protein, it suggests that it is the homodimer of MutS that probably interacts with MutL homodimer in the presence of ATP to form a repair complex.

We have observed that the presence of ATP but not ADP in the binding reaction reduces the heteroduplex-DNA binding by MutS protein. All MutS homologues contain a conserved Walker type-A P-loop motif for ATPase activity at the C-terminal region. Haber and Walker [5] demonstrated that the mutation in the conserved lysine residue in the consensus P-loop motif (GXXXGKS/T) in MutS of *Salmonella typhimurium* reduces

DNA binding. When overexpressed, this mutant had a dominant negative phenotype. Interestingly, a number of dominant negative MutS mutants of *E. coli* had mutations in the vicinity of the ATP-binding domain. Taken together, it suggests that the ATPase domain plays a very important role in mismatch recognition. Our results indicate that the ATP binding to the ATPase box but not hydrolysis reduces the DNA-binding affinity by MutS. This is drawn from our observation that ATP[S], a poorly hydrolysable ATP analogue, has greater inhibitory effect on DNA binding than ATP and non-hydrolysable ATP analogue AMPPNP has an even greater inhibitory effect on DNA binding. It may be possible that when ATP or its analogue binds to the ATPase domain on the MutS protein they change the local conformation, which affects DNA binding. But when ATP is hydrolysed by MutS, ADP and P_i are released from the protein and MutS returns back to its original conformation. Taken together, from our results it is evident that in *Taq*MutS nucleotide binding results in allosteric regulation of DNA binding similar to that which was observed in eukaryotic MutS α protein [7,10,15,18].

Limited proteolysis is a very useful tool to study the domain structure of various proteins, including *Thermus thermophilus* UvrB and MutS proteins [24–26]. The pattern of limited proteolysis by chymotryptic digestion of *Taq*MutS protein suggests that MutS is organized into a few well-defined domains in solution, consistent with the earlier observation [25]. This discrete proteolytic pattern is a characteristic of thermophilic proteins [24,25]. The patterns of the proteolytic fragmentation of MutS with heteroduplex DNA and in the presence and absence of ATP are strikingly different. This implies that extensive conformational changes occur in the MutS protein upon DNA binding followed by ATP hydrolysis, since no such changes are seen in MutS protein upon DNA binding or after ATP hydrolysis in the absence of DNA binding. At present, we do not know the regions in the MutS protein that undergo conformational changes. Further study with N-terminal sequencing of the peptide fragments generated by proteolysis will help us to understand the specific regions involved in the conformational changes. Tachiki et al. [25] recently proposed that *T. thermophilus* MutS is organized into at least four domains, A1, A2, B and C. Among these, domain B is involved in initial non-specific DNA binding followed by specific mismatch recognition by A1 and C domains. The A1 domain, which contains the F39 residue, an essential amino acid for DNA binding, interacts with the C domain containing the P-loop motif. We speculate that major conformational changes occur at the C domain upon ATP hydrolysis while MutS is bound to DNA. This change in the conformation of the C domain then results in an alteration in the DNA-binding site of MutS. In the molecular-switch model, the process of ATP binding and hydrolysis seems to switch the protein between two states in which the mismatch is recognized by the MutS–ADP complex in the ON state and not by the MutS–ATP complex, which is in the OFF state. In our case, we observed heteroduplex-DNA binding in the absence of ADP (and ATP) and that extensive conformational changes occur only in the presence of ATP and not in the presence of ADP or other cofactors. These results are consistent with the translocation model, which demands major conformational changes in the MutS–DNA complex to translocate along the DNA by utilizing ATP hydrolysis.

In addition to translocation along the DNA, it is also possible that the conformational changes in the C domain allow the MutL protein to dock on the MutS–heteroduplex complex to initiate the repair process. Interestingly, Wu and Marinus [26] have shown that the C-terminal domain including the P-loop

motif is required for MutS–MutL interaction, which suggests that conformational changes in the C domain are necessary for the interaction of the MutS–heteroduplex complex with MutL protein.

At present the three-dimensional structure of MutS protein is unknown and clear understanding of the importance of various regions of MutS, except for the C-terminal region, has not yet been gained. The MutS family of proteins from prokaryotes and eukaryotes are highly conserved. The conformational changes that we observed with *Taq*MutS may therefore represent the general properties of the MutS family of proteins. Further studies are needed with other MutS proteins along with MutL to understand the mechanism and purpose of the conformational changes in MutS protein.

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