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Structure and function of mismatch repair proteins

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Abstract

DNA mismatch repair is required for maintaining genomic stability and is highly conserved from prokaryotes to eukaryotes. Errors made during DNA replication, such as deletions, insertions and mismatched basepairs, are substrates for mismatch repair. Mismatch repair is strand-specific and targets only the newly synthesized daughter strand. To initiate mismatch repair in *Escherichia coli*, three proteins are essential, MutS, for mismatch recognition, MutH, for introduction of a nick in the target strand, and MutL, for mediating the interactions between MutH and MutS. Homologues of MutS and MutL important for mismatch repair have been found in nearly all organisms. Mutations in MutS and MutL homologues have been linked to increased cancer susceptibility in both mice and humans. Here, we review the crystal structures of the MutH endonuclease, a conserved ATPase fragment of MutL (LN40), and complexes of LN40 with various nucleotides. Based on the crystal structure, the active site of MutH has been identified and an evolutionary relationship between MutH and type II restriction endonucleases established. Recent crystallographic and biochemical studies have revealed that MutL operates as a molecular switch with its interactions with MutH and MutS regulated by ATP binding and hydrolysis. These crystal structures also shed light on the general mechanism of mismatch repair and the roles of Mut proteins in preventing mutagenesis. © 2000 Published by Elsevier Science B.V.

Keywords: MutH; MutL; Endonuclease; ATPase; Mismatch repair; Molecular switch

1. Introduction

DNA mismatch repair is an integral part of DNA replication [1]. Several processes have evolved to minimize DNA biosynthetic errors. DNA polymerase carries an intrinsic base selection and a proofreading function, which resides in the 3′–5′ exonuclease activity. Accessory proteins, such as single strand binding protein (SSB), also improve the accuracy of DNA replication. Mismatch repair is the final step to removes remaining wrongly incorporated bases (see reviews in Refs. [2–5]).

Successful mismatch repair requires two types of discrimination (see review in Ref. [6]). Firstly, it is essential to detect a mismatch in a large pool of normal base pairs. Mismatch recognition needs to detect eight different kinds of mispairs and varieties of loop structures resulting from deletion or insertion in one strand. Secondly, it is vital for the mismatch repair machinery to correct the ‘‘wrong’’ base in a

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mismatched base pair. During DNA replication, it is the newly synthesized daughter strand that contains mis-incorporated bases. Mismatch repair machinery utilizes a number of cues that distinguish a newly synthesized strand from a parental template strand. In gram-negative bacteria, strand-specific mismatch repair is methylation-dependent [7]. Due to methyltransferases, such as, deoxyadenine methyl-transferase (DAM), which adds a methyl group to the N6 of Ade in the d(GATC) sequence, template DNA is methylated, while the newly synthesized daughter strand is transiently unmodified after replication [8]. In other prokaryotes and eukaryotes, it is likely the free 3′-end of newly synthesized DNA that directs the DNA mismatch repair to that strand [3,9].

Mismatch repair proteins were first identified in *S. pneumoniae* [10]. However, the strand-specific mismatch repair system of *Escherichia coli* is the best characterized (see reviews in Refs. [3,6]). Three proteins, MutS, MutL and MutH, are sufficient to accomplish the two essential tasks: mismatch detection and targeting repair to a specific strand. MutS recognizes a mismatched basepair as well as an insertion or deletion of 1–4 nucleotides in one strand [11,12]. MutS also contains a weak ATPase activity [13], which may play roles in both mismatch recognition and signaling other proteins to assemble in the mismatch repair complex [3,13,14]. MutH is a latent sequence- and methylation-specific endonuclease. When activated, it cleaves 5′ to the unmethylated d(GATC) sequence in a hemimethylated duplex, thus targeting mismatch repair toward a daughter strand [15]. Even in the presence of a mismatch and ATP, MutS does not directly activate MutH. MutL is required to mediate the interaction between MutS and MutH [16,17]. The mismatch repair carried out by MutSLH is bidirectional and can operate over long distances; the d(GATC) site can be either 5′ or 3′ to the mismatch site and the two sites can be separated by as many as one thousand base-pairs [18]. After MutH nicks the daughter strand, DNA helicase separates the two strands and exonucleases remove the DNA from the nick to beyond the mismatch site. Finally, DNA polymerase III holoenzyme assisted by SSB protein fills in the resulting gap and DNA ligase seals the strand [19].

Homologues of MutS and MutL involved in DNA mismatch repair have been found in nearly all organisms (see reviews in Refs. [3,20]). Defective mismatch repair proteins result in genomic instability, manifested in instability of microsatellite repeats, elevated mutation rate and an increased incidence of various cancers [21,22]. In humans, the overwhelming majority of hereditary nonpolyposis colorectal cancers (HNPCC), a number of familial non-HNPCC, and sporadic cancers have been attributed to mutations in genes encoding MutS and MutL homologues [3,23,24].

In addition to their roles in replicative mismatch repair, MutL and MutS have long been implicated in both mitotic and meiotic DNA recombination and in inhibiting DNA exchange between species [3,10,25,26]. More recently, MutS and MutL homologues have also been implicated in repair of damaged DNA, such as transcription-coupled repair, and in apoptosis induced by DNA damaging agents [27,28]. Although much has been uncovered about the Mut proteins over the last 20 years, many gaps in our knowledge persist. Recent crystallographic studies of these mismatch repair proteins and biochemical analyses based on the structural information have added a new perspective to our understanding of the molecular mechanism of DNA mismatch repair.

2. Structure of MutH

E. coli MutH is a 28 Kdal, Mg^{2+}-dependent monomeric endonuclease [15]. The intrinsic endonuclease activity of MutH is very weak, but it is activated $\sim$50-fold in the presence of MutS, MutL, ATP and a mismatched basepair [29]. MutH homologues are only found in gram-negative bacteria, suggesting that different mechanisms are used for strand specificity in other organisms, such as a free 3′-end during DNA replication. In fact, the requirement for MutH in *E. coli* can be alleviated if a DNA substrate with a persistent strand break is used for mismatch repair [30]. A search of protein sequence database found that MutH is homologous to the type II restriction endonuclease *Sau*3AI [31]. Both MutH and *Sau*3AI recognize the d(GATC) sequence and cleave 5′ to the G. However, MutH cleaves an unmethylated strand either in a hemimethylated or unmodified duplex, while *Sau*3AI cleaves both strands regardless of their methylation state. When

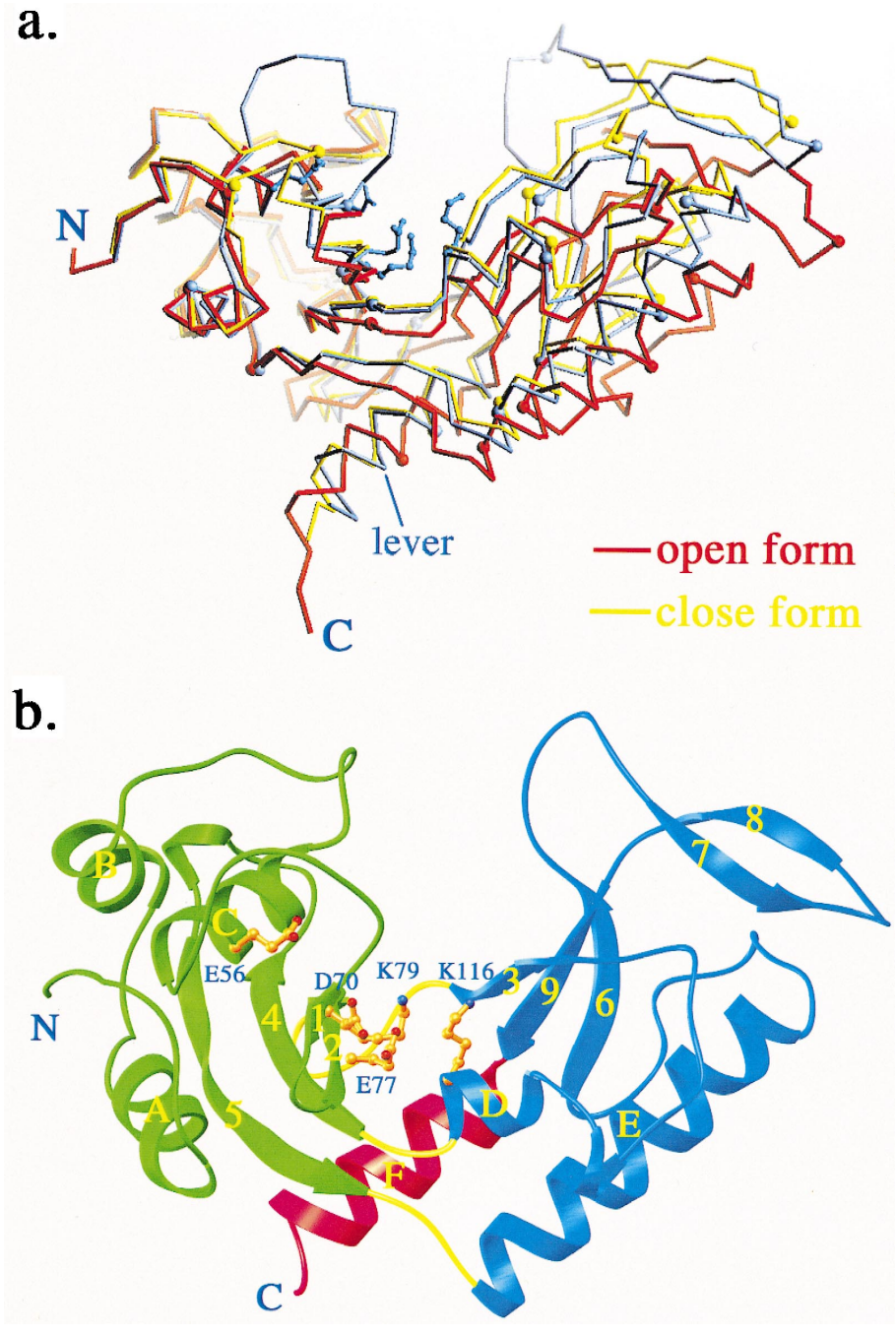

Fig. 1. Three crystal structures of *E. coli* MutH. (a) Cα trace of three independently determined crystal structures of MutH with the N-terminal subdomains superimposed. The most open form of MutH is shown in red, the most closed form in yellow and the intermediate in light blue. The five active site residues from the blue molecule are also shown. (b) Ribbon diagram of MutH in the intermediate conformation. The N-terminal subdomain is colored green and the C-terminal subdomain blue, the C-terminal ''lever'' red and linker peptides between the two subdomains yellow. The five active site residues are shown with oxygen atoms in red and nitrogen atoms deep blue.

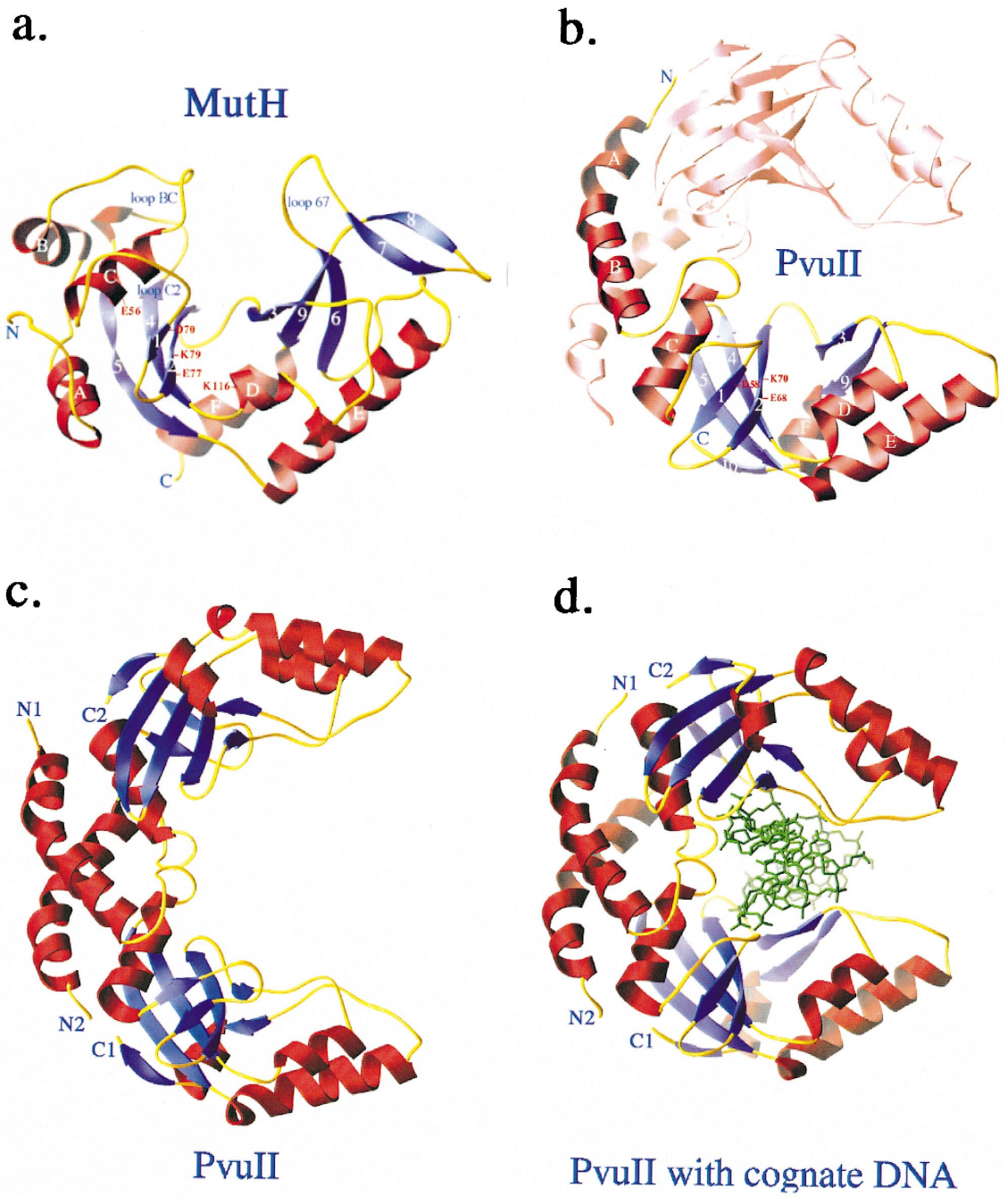

Fig. 2. Structural comparison between MutH and *Pvu*II. (a) Ribbon diagram of MutH with β-strands shown in blue and α-helices in red. (b) Ribbon diagram of the *Pvu*II protein from the *Pvu*II–DNA complex [35]. One subunit is shown with the same color scheme as MutH and the other in light pink. (c) The crystal structure of unliganded *Pvu*II [34]. The first helix (helix A) is interchanged between the two subunits, and the DNA-binding groove is wide open. (d) The crystal structure of the *Pvu*II–DNA complex [35]. *Pvu*II protein is shown in a ribbon diagram and DNA in a stick model. Upon binding of a cognate DNA, the two subunits of *Pvu*II rotate toward each other and clamp onto the DNA.

MutH makes a double strand break, it cleaves each unmodified strand independently [29,31].

Crystal structures of MutH were obtained in two different lattices at resolutions of 1.8 and 2.2 Å, respectively [31]. One of the crystal forms contains two MutH molecules in one asymmetric unit (the smallest repeating unit in a crystal). Thus, three ''snapshots'' of MutH in different crystal environments are available for structural comparison. The structure of MutH resembles a clamp with two ''arms'' separated by a large cleft (Fig. 1). All structures except three loops surrounding the central cleft are well ordered in MutH. Each ''arm'' of MutH forms a subdomain that is identical among the three independently determined MutH structures. The two ''arms'' share a hydrophobic interface and are connected by three polypeptide linkers (Fig. 1). The interface between them is apparently flexible and allows the two subdomains to pivot relative to each other (Fig. 1a).

A structural database search revealed that the tertiary structure of MutH is similar to those of type II endonucleases, such as *Pvu*II and *Eco*RV [31], although these proteins share no detectable sequence homology. Based on the structural similarity of MutH with the type II restriction endonucleases, a putative active site of MutH was identified [31]. Five residues, Glu56, Asp70, Glu77, Lys79 and Lys116 (Fig. 1b), have since been individually mutated to Ala and confirmed to be absolutely required for the endonuclease activity of MutH (M. Junop and W. Yang, unpublished data). Since these five residues are distributed on both pivoting ''arms'' of MutH (Fig. 1b), the configuration of MutH active site varies with the relative rotation between the two subdomains.

The cleft between the two subdomains in MutH also has variable dimensions due to the two pivoting ''arms''. The largest observed cleft among the three crystal structures measures 15–18 Å across and 12–14 Å deep, which is just large enough to accommodate a double-stranded DNA substrate (Figs. 1 and 2). The relative rotation between the two ''arms'' of MutH is correlated with conformational changes of the last helix (helix F) and the following residues (Fig. 1) [31]. In the most ''open'' conformation of the MutH structures, the helix F is uncoiled by one and a half turns and the residues following it are more extended. It has thus been proposed that the helix F and the following tail serve as a ''lever'' to be used to adjust the relative orientation of the two ''arms'' of MutH [31].

3. MutH is evolutionarily related to type-II restriction endonulceases

Type-II restriction endonucleases recognize short DNA sequences and in the presence of Mg^{2+}, cleave both strands of DNA within or next to a recognition site [32]. In spite of similar functions, they rarely share sequence homology. Among the crystal structures of type II restriction enzymes determined so far, conserved structural regions are limited to areas around the active site [31,33]. MutH, however, is homologous to the type II restriction endonuclease *Sau*3AI in amino acid sequence and is structurally similar to the type II restriction endonuclease *Pvu*II (Fig. 2) [31]. An interesting question arises as to how a restriction enzyme-like protein, MutH, evolved to be a regulated endonuclease that is specific for methyl-directed DNA mismatch repair. Differences between MutH and *Pvu*II-like restriction enzymes provide some clues. First of all, MutH is monomeric and makes a single-strand cleavage to initiate the mismatch repair while the active form of *Pvu*II, like most of type-II restriction enzymes, is dimeric and makes double-strand breaks. Another difference is that so far the active site of type II restriction enzymes has been found to be located in one structural domain [32] instead of two as observed in MutH. For example, there is only one structural domain in a *Pvu*II subunit and its active site is located on the N-terminal half of the protein [34,35] (Fig. 2b). The C-terminal half of *Pvu*II is much more compact than that of MutH (Fig. 2) and is tightly associated with the N-terminal domain so that they do not pivot relative to one another [31,35]. Finally, the DNA binding groove in *Pvu*II is formed between the two subunits, while it is between two subdomains in MutH (Fig. 2). However, in both proteins, the DNA binding groove exhibits ''open and close'' conformations. When *Pvu*II is bound to a cognate DNA substrate, its two subunits rotate toward each other narrowing the DNA binding groove and grip DNA tightly (Fig. 2) [34,35].

MutH becomes a monomeric endonuclease, different from type II restriction enzymes, by enlarging

the C-terminal half of the molecule such that it gains a second structural domain and develops a DNA binding groove within a single subunit (Fig. 2a). MutH also acquires additional residues to form an active site, for example, Lys116 has no counterpart in *Pvu*II. Moreover, both the substrate-binding groove and the active site configuration are subject to changes. It is likely that through the C-terminal ''lever'', MutL and MutS help MutH to orient the two subdomains to receive DNA substrate and to configure its active site appropriately. The MutH-homologous restriction endonuclease *Sau*3AI, which is independently active, contains additional 270 residues at its C-terminus that may serve to alleviate the requirement of MutS and MutL for activation as MutH does [31].

4. Structure of the ATPase domain of MutL

MutL and its homologues form a large protein family with members found in species ranging from archaebacteria to mammals [3,36]. All of the MutL family members share extensive sequence homology in the N-terminal 300–400 residues, while the C-terminal half of their sequences are very diverse. All members of the MutL family characterized so far form homo or heterodimers via the C-terminal region [37–39].

Initially, MutL was not thought to possess an enzymatic activity [3]. When the structure of an N-terminal 40 Kdal fragment of MutL (LN40), which encompasses all conserved residues in the MutL family, was determined, it was thus a surprise to discover that it is structurally similar to the ATPase fragment of DNA gyrase [40]. However, several lines of evidence now support that MutL contains a genuine ATPase activity. First, MutL can activate the endonuclease activity of MutH only in the presence, but not in the absence, of ATP [39]. Second, sequence alignments by Bergerat et al. [41] and Koonin's group [42] independently suggested that three non-Walker-like sequence motifs involved in ATP binding and hydrolysis in DNA gyrase (a member of the type II topoisomerase family) are also conserved in the MutL and Hsp90 families. Third, MutL does contain an intrinsic although low ATPase activity of 0.4/min [39]. When the residue in the MutL equivalent to the general base for ATP hydrolysis in DNA-gyrase (Glu29) is mutated to Ala, MutL loses this ATPase activity completely [39,43]. Four, inhibitors of the ATPase activity of DNA gyrase, such as novobiocin and coumarin, also inhibit the ATPase activity of MutL [39].

LN40 consists of two domains, residues 20–200 form the first domain and residues 207–331 form the second domain, both of which are of mixed $\alpha\beta$ folds (Fig. 3a) [39]. In addition to the ATPase domain of DNA gyrase, the first domain of MutL is homologous to the N-terminal 20 KDal fragment of Hsp90, which can bind ADP and an anticancer drug, geldanamycin [44,45]. Whether Hsp90 contains ATPase activity was an issue causing controversy for many years. Parallel to the characterization of the ATPase activity of MutL, the ATPase activity of Hsp90 was finally and definitively confirmed using similar approaches to studies of MutL [46,47]. Thus, type II topoisomerase (Gyrase), Hsp90 and MutL protein families form a new ATPase superfamily (GHL superfamily), unrelated to those containing Walker-A boxes [39].

In the absence of nucleotide ligand, the structure of LN40 is monomeric and partially unstructured [39]. Nearly 20% of its residues are disordered in the crystal structure (Fig. 3a), many of which are conserved in the MutL family and presumably important for function. Even though, the overall structure of LN40 bears a strong resemblance to that of the ATPase domain of DNA gyrase, the ATP binding pocket of LN40 is considerably different from that of DNA gyrase [39]. The structure of the ATPase fragment of DNA gyrase was determined in the presence of ADPnP (a non-hydrolyzable analog of ATP) [40]. In the absence of a ligand, the ATP-binding pocket of LN40 is occupied by protein side chains, which must move to accommodate ATP [39].

5. Structural transformation of MutL by ATP binding and hydrolysis

Solution studies indicate that binding of a non-hydrolyzable ATP analog (ADPnP) transforms LN40 from monomeric to dimeric [39]. Crystal structures of LN40 complexed with ADPnP and ADP have been separately determined [43]. In both structures,

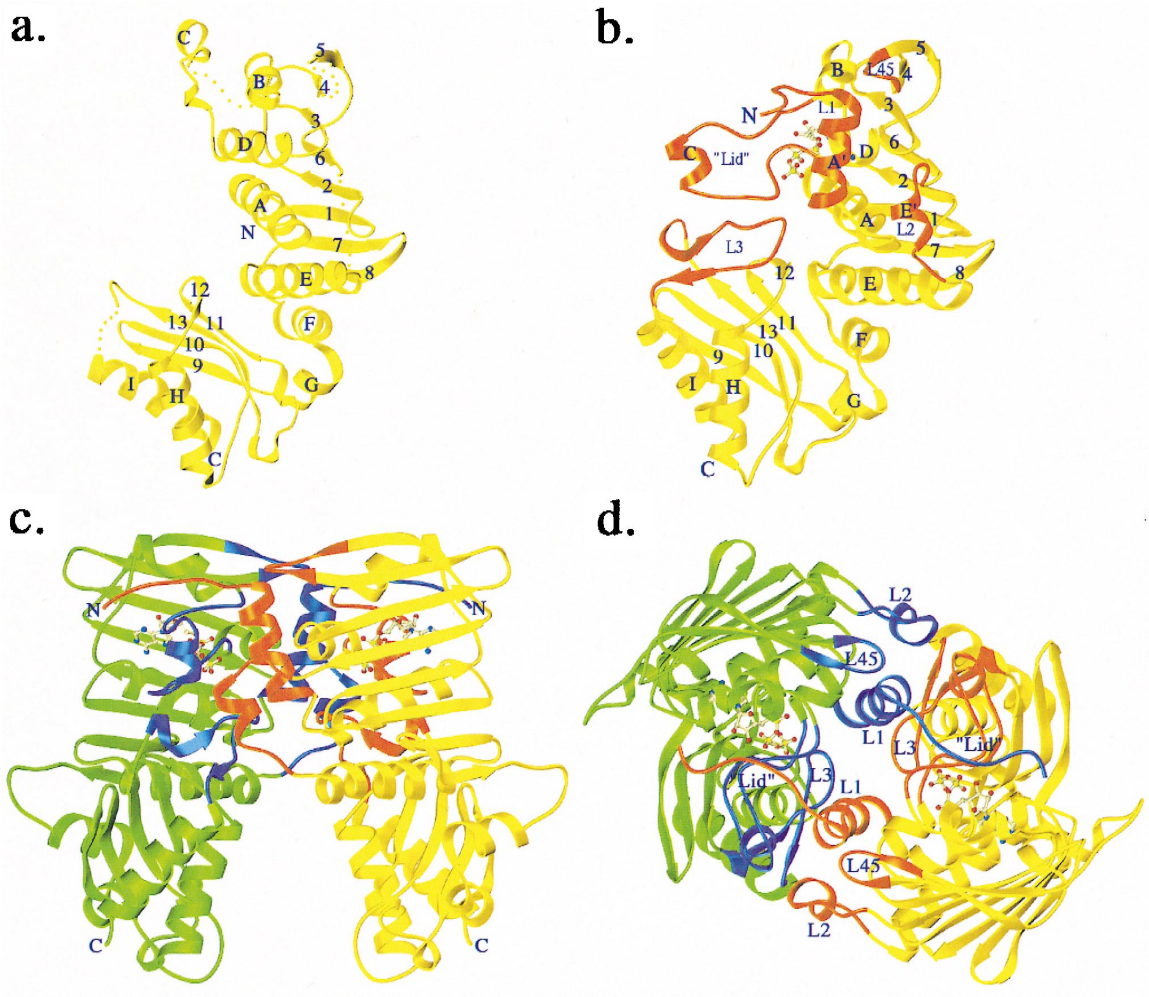

Fig. 3. The crystal structures of LN40 and LN40–ADPnP complex. (a) Ribbon diagram of monomeric LN40. Secondary structures are labeled sequentially, α-helices from A to I and β-strands from 1 to 13. Four internal disordered loops are represented by dotted lines. The boundary between the first and second domain is found at the end of strand 8 and beginning of helix F. (b) Ribbon diagram of one subunit of the LN40–ADPnP dimer. The nucleotide is shown in a ball-and-stick model. Structures that were disordered in (a) are shown in red. The elbow angle between the two domains is more acute than that in (a) because loop L3 is hydrogen bonded to the γ-phosphate. (c and d) Orthogonal views of the LN40-ADPnP dimer. One subunit is shown in green and blue and the other in yellow and red. Blue and red represent newly formed secondary structures, all of which are involved in dimer interactions, three of which (L1, Lid and L3) are also involved in ATP binding.

LN40 indeed becomes dimeric. Five loops disordered in the apo-protein structure, which encopass nearly 70 residues, become well ordered in the LN40-ADPnP complex (Fig. 3b). Roughly one third of these re-organized residues are directly involved in nucleotide binding, and the rest of them are essential for dimer formation. The ADP moiety is bound entirely within the first domain of LN40. Four conserved sequence motifs shared in the GHL superfamily are responsible for adenine recognition and for binding of the α- and β-phosphate [43]. The γ-phosphate is coordinated by residues from both the first and the second domains. The presence of a γ-phosphate re-orients the two domains and brings the second domain closer to the first (Fig. 3a and b). When ATP is hydrolyzed, such as in the LN40-ADP

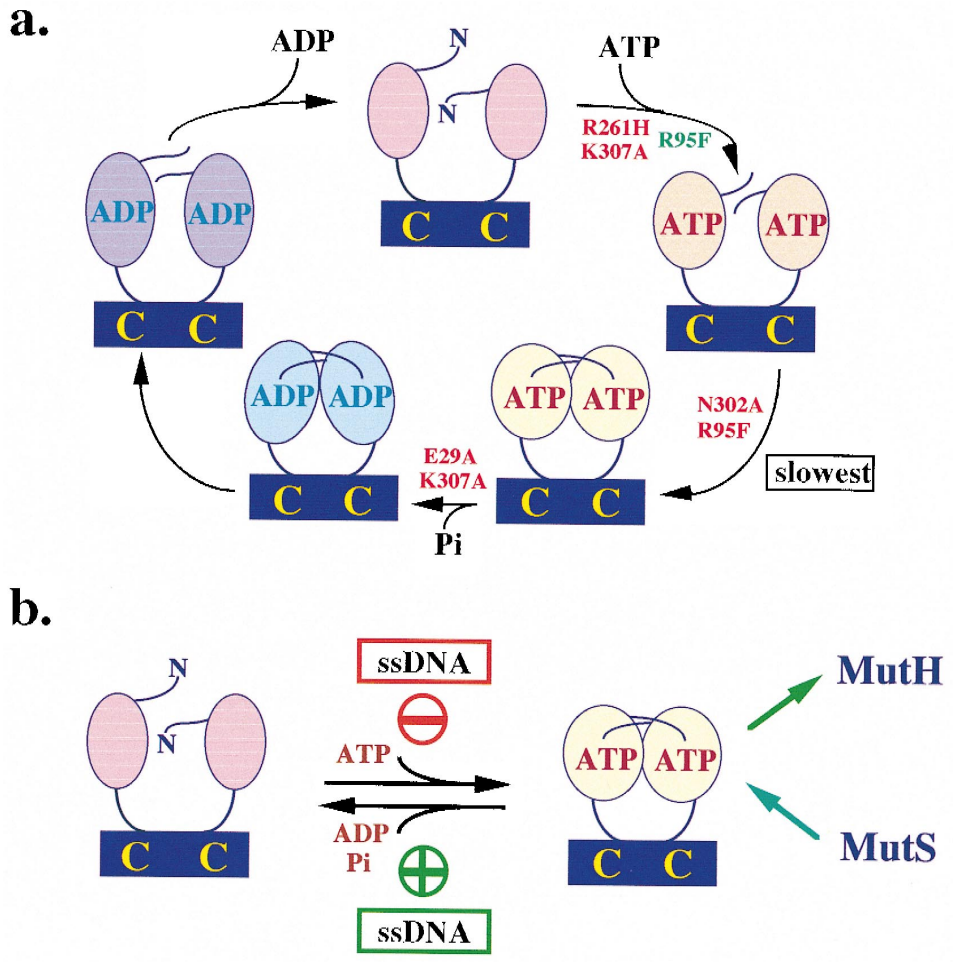

Fig. 4. Diagram of the MutL ATPase cycle. (a) Discrete structural states have been identified combining structural, biochemical and mutagenic studies. Mutations that inhibit structural transformation are labeled in red; those that enhance transformation are in green. Three of the states (apo–protein, dimeric LN40–ADPnP and LN40–ADP complex) have been observed in crystal structures. (b) The MutL ATPase cycle is simplified to two states. The presence of ssDNA favors the nucleotide free state of MutL by increasing both Km (reducing ATP binding) and kcat (enhancing ATP hydrolysis). Interactions between MutL and MutH depend on association of ATP and MutL. Mismatch- and MutS-dependent activation of MutH requires the complete ATPase activity of MutL.

complex, the γ-phosphate diffuses away and the second domain is released from the first [43]. In solution, the LN40-ADP complex dissociates to become monomeric and quickly releases ADP [39,43]. The structural transformation brought about by the nucleotide greatly alters the appearance of LN40. Similar changes occur in the full-length MutL as indicated by solution studies [39]. Supplemented by characterization of various MutL mutants defective in successive steps of ATP binding and ATP-hydrolysis, the crystal structures of LN40 can be incorporated as distinct states into an ATPase cycle as depicted in Fig. 4 [43].

6. Interactions among MutL, MutS and MutH

The first piece of evidence that binding of a nucleotide to MutL regulates protein–protein interactions came from the observation that MutL alone can activate MutH in an ATP-dependent manner [39]. Further investigation of the effects of non-hydrolyza-

ble analogs of ATP on MutH activation indicates that ATP-binding and not hydrolysis by MutL is essential for activating MutH [39]. In addition, MutL mutants that retain ATP-binding but are defective in ATP hydrolysis activate MutH well; MutL-ADP complexes are also able to activate MutH but at a lower level (Junop and Yang, unpublished data). Apparently, activation of MutH does not require the presence of a γ-phosphate. However, without a γ-phosphate, the MutL-ADP complex is less stable and falls apart more readily [39]. Indeed, MutL bound with ADPnP rather than ATP is better able to activate MutH [39], and MutL mutants defective in ATP-hydrolysis activate MutH better than the wild-type protein (Junop and Yang, unpublished data). Presumably, in both cases the MutL-nucleotide complexes are more stable and have a longer half-life.

Physical interactions between MutH and MutL are evidenced both by in vitro protein–protein cross-linking studies and in vivo two-hybrid experiments [39,48]. Guided by the protruding C-terminal ''lever'' of MutH and the requirement of nucleotide–MutL association for the interactions, we modeled the interactions between MutH and the LN40–ATP complex. By positioning the C-terminal ''lever'' of MutH, which protrudes from a convex surface, toward a concave surface of MutL, where the adenine base is bound, the resulting model of MutL–MutH complex shows good shape complementary (Fig. 5). This model, although plausible, awaits genetic and biochemical testing!

The interactions between MutL and MutS, unlike those between MutL and MutH, are more elusive. So far, MutL and MutS have been only co-localized to the same piece of DNA by immuno-precipitation [49]. Normally, cleavage of a DNA containing both a mismatch and d(GATC) sequence by MutH is increased by MutL in the presence of ATP and is further stimulated by addition of MutS. Interestingly, the MutL mutants, which are normal in ATP binding but defective in hydrolysis, fail to further stimulate MutH after addition of MutS (Junop and Yang, unpublished data). Apparently, the ATPase activity of MutL is essential for MutL to interact with MutS and fully activate MutH in a mismatch-dependent fashion.

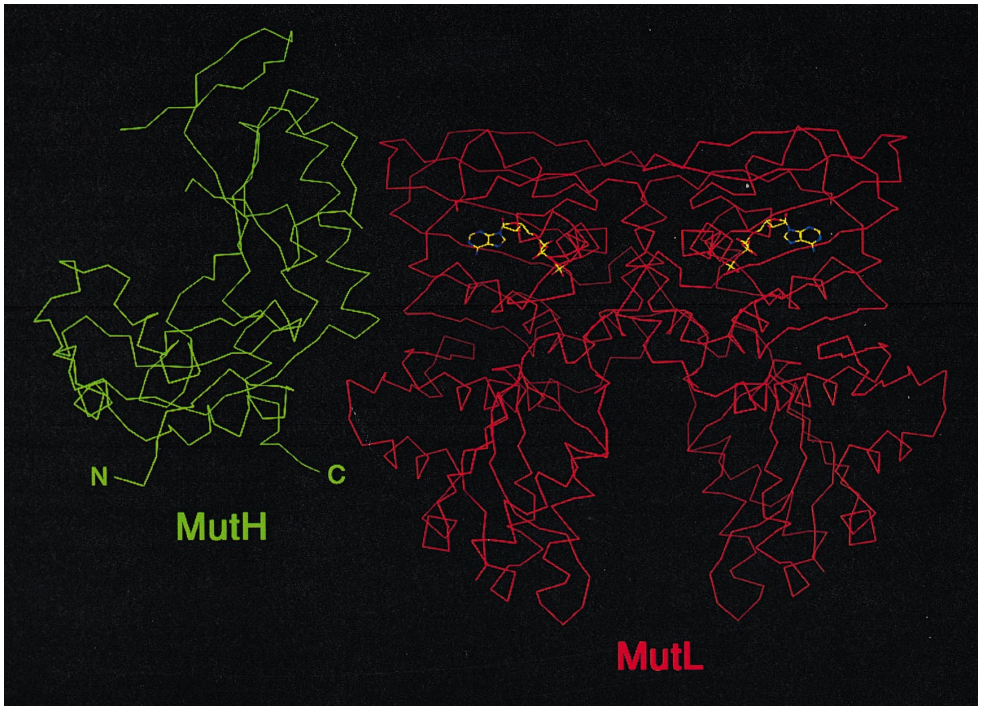

Fig. 5. A molecular model of interactions between MutL and MutH.

7. MutL is a molecular switch in DNA mismatch repair

Proteins that hydrolyze nucleotide triphosphate are generally categorized into two classes: motor proteins like myosin, actin or DNA helicase, and signaling proteins, such as G-proteins. An interesting structural and functional comparison between the two classes has been made [50] and guides characterization of MutL. MutL does not seem to be a motor protein for the following reasons. MutL hydrolyzes ATP about 100-fold more slowly than any known motor protein [39]. Although it binds to DNA, which in turn activates its ATPase activity, MutL does not possess a helicase activity (Junop and Yang, unpublished data). Its function in mediating interactions between MutS and MutH, however, suggests that MutL is an ATP-operated signaling molecule.

At least two criteria have to be fulfilled for the ATPase activity to be utilized by MutL for signaling. Firstly, different states of the nucleotide–MutL complex must be used to interact with different partners. In addition to activating MutH, MutL is known to interact with the DNA helicase UvrD [38,51] and plays a role in recruiting DNA polymerase III to complete the repair process [3] after MutH nicks the daughter strand. Eukaryotic MutL homologues have been shown to interact with replication protein PCNA [52] and exonuclease I (personal communication with R. Fishel). The participation of these MutL-contacting proteins in the mismatch repair process is temporally separated. Secondly, the ATPase cycle of MutL must be regulated, perhaps by more than one cofactor, so that one structural species of MutL is dominant at a particular time and serves a specific function. We found that DNA, single-stranded in particular, stimulates the ATPase activity of MutL [43]. Using site-specific mutagenesis, the deep cleft formed between the two subunits in the LN40–ADPnP structure (Fig. 3c) was found to be the site where LN40 interacts with DNA [43]. Careful analyses of the kinetic effects of ssDNA on the MutL ATPase activity revealed that both Km and kcat are increased, meaning that ssDNA lowers the affinity of MutL for ATP while enhancing the rate of ATP hydrolysis [43]. Thus, ssDNA changes the distribution of MutL among different structural states and favors the nucleotide-free form, which does not activate MutH. During the mismatch repair process, ssDNA occurs after MutH nicks the daughter strand and exonuclease starts to remove nucleotides and exposes the template DNA. At that stage, MutH is no longer needed, while helicase and DNA polymerase should be recruited. Controlled by ATP binding and hydrolysis, MutL thus may serves as a molecular switch that recruits different proteins at various steps in the mismatch repair process. In addition, the involvement of MutS, MutL and their homologues in repair of oxidative or carcinogen damaged DNA and the connections between mismatch repair and programmed cell death [28,53–55] strongly suggest that the requirement of a molecular switch or adapter to coordinate various DNA repair pathways and to choose repair versus apoptosis. MutL seems to be a promising candidate to fulfill such a requirement.

8. Mismatch repair, mutagenesis and cancers

Analyses of MutL mutants defective in mismatch repair confirm the importance of the ATPase activity of MutL and correlate well with the prediction that MutL is a molecular switch. Most point mutations in MutL that cause the dominant mutator phenotype in *E. coli* are located in or near the ATP binding pocket [43,56]. These ATPase defective mutants can cripple the mismatch repair process at two levels. As discussed earlier, MutL mutants defective in ATP-hydrolysis but not ATP-binding activate MutH better than the wildtype MutL, which may cause MutH to cleave DNA independent of mismatch detection thus interfering with normal DNA replication. Moreover, MutL mutants that lack ATPase activity fail to mediate between MutS and MutH (see Section 6) and block the mismatch repair pathway completely. The majority of missense mutations found in homologs of MutL in HNPCC kindreds are located around the ATP-binding pocket [24,43], which further corroborates the linkage between a defective molecular switch and mutagenesis in the cell.

9. Conclusions

Crystal structures are often instrumental in elucidating mechanism of a biological process. In this

case, the structures of MutH and LN40 have led to identification of the active site of MutH, the ATPase activity of MutL, the regulated interactions among MutS, MutL, and MutH, and a more detailed mechanistic understanding of mismatch repair. However, the more we learn, the more unknowns we encounter. We do not know how MutH distinguishes an unmethylated from a modified d(GATC) sequence. We have not determined the structure of the C-terminal portion of MutL and still lack structural information on the mismatch recognition protein MutS. We ask what functions the ATPase activity of MutS serves and whether and how the MutL and MutS ATPases interact with one another. Crystal structures, including each individual protein, protein–protein and protein–DNA complex, are certain to prove invaluable for unraveling the mechanism of these complicated and interesting proteins.

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