Escherichia coli mismatch repair protein MutL interacts with the clamp loader subunits of DNA polymerase III

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Abstract

It has been hypothesized that DNA mismatch repair (MMR) is coupled with DNA replication; however, the involvement of DNA polymerase III subunits in bacterial DNA MMR has not been clearly elucidated. In an effort to better understand the relationship between these 2 systems, the potential interactions between the *Escherichia coli* MMR protein and the clamp loader subunits of *E. coli* DNA polymerase III were analyzed by far western blotting and then confirmed and characterized by surface plasmon resonance (SPR) imaging. The results showed that the MMR key protein MutL could directly interact with both the individual subunits δ, δ', and γ and the complex of these subunits (clamp loader). Kinetic parameters revealed that the interactions are strong and stable, suggesting that MutL might be involved in the recruitment of the clamp loader during the resynthesis step in MMR. The interactions between MutL, the δ and γ subunits, and the clamp loader were observed to be modulated by ATP. Deletion analysis demonstrated that both the N-terminal residues (1–293) and C-terminal residues (556–613) of MutL are required for interacting with the subunits δ and δ'. Based on these findings and the available information, the network of interactions between the MMR components and the DNA polymerase III subunits was established; this network provides strong evidence to support the notion that DNA replication and MMR are highly associated with each other.
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Keywords: DNA polymerase III; Mismatch repair; MutL; Clamp loader; Interactions

1. Introduction

The mismatch repair (MMR) system is the primary pathway for correcting DNA replication errors. Defects or inactivation of MMR genes results in genomic insta-bility and increases spontaneous mutability by 50- to 1000-fold [1]; this subsequently results in a number of problems such as drug resistance [2] and human cancers [3–5].

Much of our current understanding of MMR comes from studies on *Escherichia coli* methyl-directed MMR that involves the proteins MutS, MutL, MutH, UvrD, etc. According to an existing model [6–8], MutS recognizes and binds DNA mismatches, and this binding triggers the subsequent steps of the MMR pathway, including the activation of MutH by MutL together with

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MutS and ATP, which cleaves the transiently unmethylated daughter strand at hemimethylated d(GATC) sites, unwinding of dsDNA by UvrD, excision of ssDNA by several exonucleases, gap filling by resynthesis of DNA by DNA polymerase III, and finally ligation of the nick by DNA ligase.

It has been established that DNA polymerase III plays a major role in DNA replication [9,10], and the gap produced by MMR is filled in by DNA polymerase III. Further, the DNA polymerases (e.g., DNA polymerase I) are employed in other repair pathways (e.g., nucleotide excision repair) and are not necessarily required for normal DNA replication [11]. These facts indicate that MMR is closely related to DNA replication.

DNA polymerase III consists of the following 10 subunits: β subunit (β clamp), clamp loader (complex of subunits δ, δ′, γ, Ψ, χ), τ subunit, and the core enzyme (complex of subunits α, θ, ε) [12]. In the eukaryotic MMR system, proliferating cell nuclear antigen (PCNA, homologue of β subunit), DNA polymerase δ, and the replication factor C (RFC) clamp loader (homologue of the clamp loader of *E. coli* DNA polymerase III) play important roles in the regulation of mismatch-provoked excision [13,14]. Nevertheless, the involvement of *E. coli* DNA polymerase III subunits in the mismatch recognition, excision, and resynthesis steps of MMR have not been clearly addressed [6].

In order to thoroughly understand the MMR process, extensive investigation of the interactions of the individual components of MMR and DNA polymerase III is required. Among these components, the interactions between the β subunit of DNA polymerase III and MutS or MutL have been documented [15]. The function of the clamp loader is partially known; it can load the β subunit onto DNA during replication, indicating that the clamp loader of the polymerase III holoenzyme is required for methyl-directed MMR *in vitro* [6,16,17]. Since the functional clamp loader comprises a minimum of 3 subunits, i.e., δ, δ′, and γ [18], these subunits are normally selected for *in vitro* experiments [19,20].

In the current study, we focused on the interactions between the *E. coli* MMR key protein MutL and the DNA polymerase III clamp loader subunits. Surface plasmon resonance (SPR) imaging, a method that enables the study of molecular interactions [21], and far western blot analysis were adopted to evaluate the interactions. Our results together with those of previous reports enabled us to determine the protein-interaction network between the MMR system and DNA polymerase III.

2. Materials and methods

2.1. Materials

All chemicals were purchased from Sigma Chemicals Company. Restriction enzymes, DNA polymerase (Pyrobest), and T4 DNA ligase were from TaKaRa Company. The PCR purification mini kit and gel extraction mini kit were from Omega Company. TALON^{TM} metal affinity resin was from Novagen Company. Milli-Q water was used in all assays.

2.2. Plasmid construction

The genes *holA* (coding for the δ subunit), *holB* (coding for the δ′ subunit), *dnaX* (coding for the truncated γ subunit), and *mutL* (coding for MutL) were amplified from *E. coli* K-12. Each of the genes was subcloned into pQE30 (Qiagen). The strains used to express UvrD and glutathione *S*-transferase (GST) were stored in our lab. pQE30-*mutL-sbp* was constructed as follows: a streptavidin-binding peptide (SBP, MDEKTTGWRGGHVVEGLAGELEQLRAR LEHH-PQGQREP) coding sequence together with a linker peptide (Gly$_3$-Ser$_3$-Gly$_3$)$_3$ coding sequence was amplified from the plasmid pTAG2K (Gene bank ID: 14211969, kindly provided by David S. Wilson, Howard Hughes Medical Institute) and was inserted into the C-terminus of *mutL*. *mutL-sbp* was also subcloned into pET15b (Novagen). The *mutL* gene in pQE30-*mutL-sbp* was deleted by using the cloning strategy outlined above; the different deletion MutL-SBP mutants are listed in Fig. 5(A). All clones were confirmed by DNA sequencing.

2.3. Protein expression and purification

Except for GST, all proteins were expressed in *E. coli* M15 cultured in Luria–Bertani (LB) medium containing 100 μg/ml ampicillin (Amp) and 35 μg/ml kanamycin (Kan). GST was expressed in *E. coli* BL21 (DE3) in LB medium containing 100 μg/ml Amp. The cells were cultured at 37 °C. When the absorbance ($\lambda = 600$ nm) of the cultures reached 0.6, the cells were induced by addition of 0.4 mM isopropyl thio β-D-galactoside (IPTG) in order to express the δ′ subunit and MutL or MutL mutants at 30 °C for 4 h, respectively. The recombinant strains expressing UvrD, the γ subunit, and the δ subunit were induced by IPTG at 25 °C for 5 h to avoid the formation of an inclusion body. Cells were harvested by centrifugation at 4000 rpm for 20 min and then lysed by ultrasonication. Cell fragments were separated by centrifugation at 12,000 rpm for 30 min at 4 °C. Most of the proteins were purified using the Ni^{2+}-NTA column (Amersham) and GST was purified using sepharose 4B (Amersham). Their purities were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The δ′ subunit, γ subunit, GST, and the MutL and MutL mutants were stored in buffer A (25 mM Tris–HCl, pH 7.6; 150 mM NaCl, 1 mM 1,4-dithiothreitol (DTT) and 5% of glycerol), UvrD was stored in buffer A containing 10% of

glycerol, while the δ subunit was stored in buffer A containing 20% of glycerol. *In vitro* assembly of the clamp loader complex was carried out according to a previously described method [19]. Briefly, the subunits δ, δ′, and γ were mixed in the ratio 1:1:1.5 at 4 °C in buffer B (50 mM Tris–HCl, pH 7.6, 100 mM NaCl, 1 mM DTT and 10% of glycerol) overnight. In order to purifying the clamp loader, the mixture was loaded onto a 1 ml Fast-flow Q (FFQ) column (Amersham) and a 1 ml Resource Q column (Amersham) sequentially and eluted with a linear gradient of NaCl (50–500 mM); the fractions containing the clamp loader complex were pooled. To achieve a purity greater than 95%, the pooled proteins were further purified by gel filtration using a Superdex 200 column (Amersham).

2.4. Bioactivity determination of MutL-SBP

A complementation assay based on the mutator phenotype was designed to verify the bioactivity of the fused MutL-SBP structure. Cells lacking a functional chromosomal *mutL* gene showed a mutator phenotype that was analyzed by the frequency of occurrence of rifampicin-resistant clones. Single colonies of *mutL*-deficient KM52 cells (*mutL⁻*, kindly provided by Dr K.C. Murphy, University of Massachusetts Medical School) or the cells transformed with the pET15b-*mutl-sbp* vector were grown overnight at 37 °C in 5 ml of LB medium. Aliquots of 50 μl from the undiluted culture were plated on LB agar containing 100 μg/ml rifampicin with or without 100 μg/ml Amp. Colonies were counted following overnight incubation at 37 °C.

2.5. Far western analysis

Four concentrations – 200, 100, 50, and 25 nM – of each of the 3 target proteins δ, δ′, and γ subunits; the positive control UvrD; and the 2 negative controls BSA and GST were separately loaded onto a nitrocellulose membrane from left to right. The membrane was blocked for 2 h at room temperature with buffer C (25 mM Tris–HCl (pH 7.6), 150 mM NaCl, 5% (w/v) non-fat milk, and 1 mM EDTA) and then incubated with 400 nM MutL-SBP in buffer C for 2 h at 37 °C, followed by successive washing 3 times each with buffer D containing 25 mM Tris–HCl (pH 7.6), 150 mM NaCl, 0.05% Tween 20, and buffer D without Tween 20. In order to assay the interactions between MutL and the target proteins, alkaline-phosphatase conjugated strepavidin (Promega) at a 1:3000 dilution in phosphate buffered saline (PBS) buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na₂HPO₄, and 2 mM KH₂PO₄, pH 7.4) was added onto the membrane. The enzyme was bound to the membrane through the binding of its fusion partner streptavidin to the SBP (the fusion partner of MutL). After 1 h incubation, the membrane was washed 3 times with buffer D (without Tween 20). The enzyme substrate 5-bromo-4-chloro-3-indolylphosphate/nitroblue tetrazolium (BCIP/NBT) (DingGuo) was then added; the development of a purple color indicated a positive reaction.

To assay the different MutL-SBP deletion mutants, three concentrations, i.e., 200, 100, and 50 nM of each of the subunits δ, δ′, and γ and BSA were loaded onto different nitrocellulose membranes from left to right. The assay was conducted in a manner similar to that described above except that MutL-SBP was substituted by 400 nM of a different deletion mutant of MutL-SBP.

2.6. SPR experiments

SPR experiments were carried out on a BIAcore 3000 machine (BIAcore AB, Uppsala, Sweden) at 25 °C. The BIAcore 3000 system contains a dual-channel measuring cell; the working channel is linked to a sensor chip while the reference channel is linked to the same chip without the immobilized sensor element protein. The carboxymethylated dextran surface-modified chip (CM5 chip) was employed to prepare the δ′ subunit sensor chip and the γ subunit sensor chip according to the amine-coupling protocol of the BIAcore manual. In addition, the streptavidin-modified chip (SA chip) was used to immobilize MutL-SBP in a specific orientation via the SBP–strepavidin interaction. Before measurement, the sensor chips were equilibrated with running buffer at the rate of 30 μl/min. The running buffer contained 25 mM Tris–HCl (pH 7.6), 150 mM NaCl, 1 mM DTT, 5 mM MgCl₂, and 0.005% (v/v) Tween 20. Samples were injected at different concentrations at a flow rate of 30 μl/min for 2 or 3 min. The regeneration of the sensor chip surface was carried out according to Li et al. [22].

The curves were fitted to a 1:1 Langmuir binding model (BIAevaluation 4.1 software) to obtain the equilibrium and kinetic constants.

3. Results

3.1. Full-length MutL can specifically interact with the subunits δ, δ′, and γ directly according to the far western assay

As shown in Fig. 1, on the LB plates containing 100 μg/ml rifampicin, hundreds of KM52 colonies were visible on the plate, while few colonies of KM52/pET15b-*mutl-sbp* were observed. Since *E. coli* KM52 is *mutL* deficient, it is presumed that the gene *rpoB* (rifampicin target) is prone to mutate at a much higher frequency than the wild-type strain [23]; this is probably responsible for the induced rifampicin resistance. The introduction of extrinsic *mutL* (*mutL-sbp*) provided the strain with requisite MutL activity so that it is sensitive to rifampicin. MutL-SBP can function in *vivo*, and it can be inferred that MutL-SBP retains most of the bioactivity of MutL. Thus, purified MutL-SBP is suitable to investigate potential protein interactions in *vitro*.
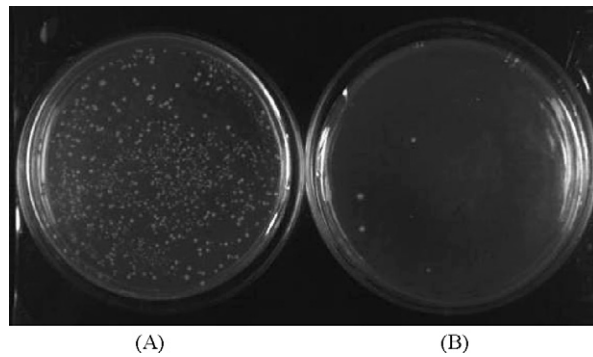
Fig. 1. Complementation assay based on the mutator phenotype. Cultures of (A) KM52 (*mutL⁻*) and (B) KM52/pET15b-*mutl-sbp* were grown at 37 °C for 12 h under the appropriate antibiotic selection. Fifty microlitres of culture was then plated on LB plates containing 100 g/ml rifampicin with or without 100 mg/ml ampicillin, respectively, and incubated overnight at 37 °C. This experiment had been repeated for three times.

Fig. 2. Far Western analysis of interactions between MutL and subunits δ, δ′ and γ. δ, δ′, γ, UvrD and BSA or GST were applied to a nitrocellulose membrane with different concentrations (200–25 nM, from left to right) and then incubated with MutL-SBP. UvrD was set as positive control and BSA and GST were set as negative control, respectively. MutL-SBP remained bound to the membrane was detected by incubating with streptavidin alkaline phosphatase conjugate and BCIP/NBT sequentially.

Direct interactions between MutL and the subunits δ, δ′, and γ were observed in both the far western blot and SPR analysis. Fig. 2 shows the result of the far western blot analysis. UvrD is known to interact with MutL [24] and exhibited a positive result in this experiment, while BSA and GST showed a negative result. Positive signals were also observed in the area of the membrane where the subunits δ, δ′, and γ had bound, indicating that these subunits can directly interact with MutL. In order to validate the newly found interactions and quantify the kinetic parameters of the interactions, SPR was adopted for further analysis.

3.2. SPR analysis confirmed the interactions between MutL and subunits δ, δ′, and γ

The MutL-modified SA chip was used to study the interactions between MutL and the δ subunit, while the δ′ subunit- or γ subunit-modified CM5 chip were selected for studying the interactions between MutL and the subunits δ′ and γ, due to weak non-specific adsorption of the subunits δ′ and γ on the SA chip. Clearly, as shown in

Fig. 3, there were interactions between MutL and the 3 subunits. The response unit (RU) values were proportional to the sample concentrations in certain ranges. The K_D values of the interactions between MutL and the subunits δ, δ′, and γ were 4.08, 2.41, and 1.85 nM (Table 1), respectively, showing similar affinity. All three sensorgrams exhibited fast association and slow dissociation rates, indicating that it was difficult to dissociate the MutL bound to these subunits. Similar positive and negative controls were used in the far western analysis and the SPR assay. Both BSA and GST did not interact with MutL, while UvrD exhibited a strong interaction signal (Fig. 3D); this result is consistent with that of the far western experiment.

Table 1
Kinetic constants of interactions between MutL and clamp loader subunits

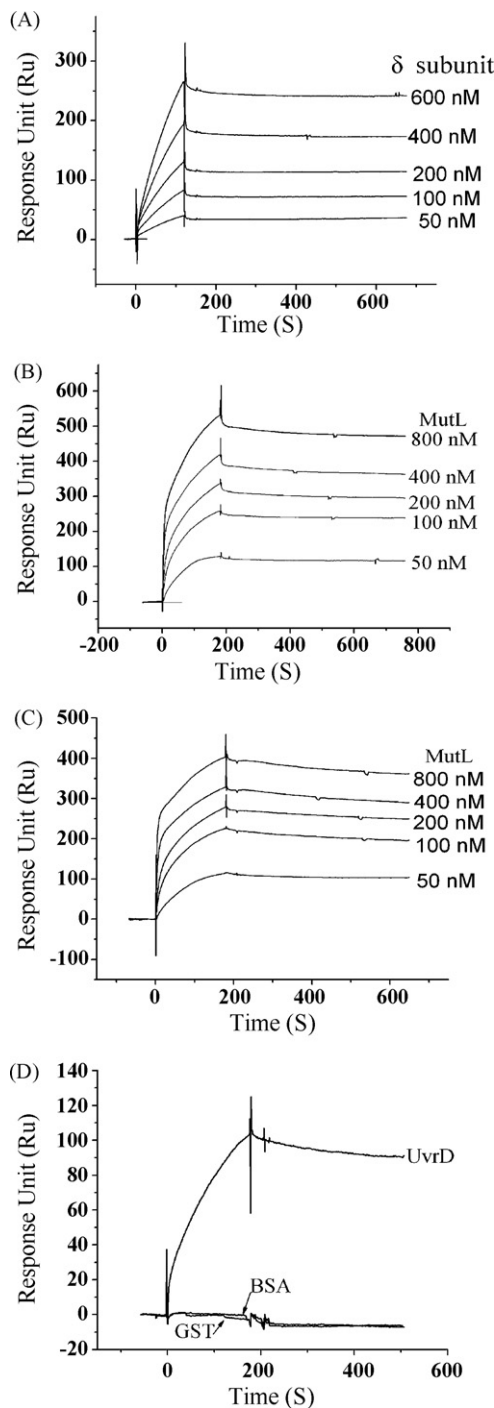| Interaction	K_a (M⁻¹ S⁻¹)	K_d (S⁻¹)	K_A (M⁻¹)	K_D (M)
δ subunit and MutL	1.33×10^4	5.42×10^{-5}	2.45×10^8	4.08×10^{-9}
δ′ subunit and MutL	4.86×10^4	1.16×10^{-4}	4.17×10^8	2.41×10^{-9}
γ subunit and MutL	1.11×10^5	2.05×10^{-4}	5.40×10^8	1.85×10^{-9}

Each experiment has been repeated for three times.

Fig. 3. SPR sensorgrams showing the interactions between MutL and subunits δ, δ′ and γ. (A) Immobilized phase: 50 nM MutL-SBP fusion (on SA chip), injection sample: δ subunit with the indicated concentrations (flowing for 120 s); (B and C), immobilized phase: 50 nM δ′ subunit and γ subunit, respectively (on CM5 chip), injection sample: MutL with the indicated concentrations (flowing for 180 s); (D) immobilized phase: 50 nM MutL-SBP fusion (on SA chip). Injection sample: 200 nm UvrD, BSA and GST respectively (flowing for 180 s). This experiment had been repeated for at least three times.

3.3. Interactions between MutL and the δ subunit, γ subunit, and clamp loader were modulated by ATP

MutL is an ATPase, and ATP hydrolysis is critical for the functioning of MutL as it enables this protein to modulate the activities of a few other protein players involved in MMR [25]. For example, in the presence of ATP, MutL can interact with the MutS–ATP–DNA complex and activate the latent endonuclease activity of MutH [26]. In SPR experiments, the interactions between MutL and the δ subunit, γ subunit, and clamp loader were weakened to a great extent by the presence of 1 mM ATP or AMPPNP (analog of ATP) (Fig. 4). The inhibition effect of AMPPNP was stronger than that of ATP in these cases, and the interactions were almost abolished on the addition of AMPPNP (Fig. 4A and B). This is because AMPPNP is an ATP analog that can bind to the ATPase site but cannot be hydrolyzed. This process appeared very similar to the control of the G protein molecular switches by GDP → GTP exchange. However, the interaction between MutL and the clamp loader in the presence of ATP or AMPPNP is considerably less pronounced. The multisubunit clamp loader couples ATP binding and hydrolysis in order to assemble the sliding clamp around DNA at a primed site; however, with the subunits δ or γ alone, this was not possible [18]. Since both MutL and the clamp loader can bind and hydrolyze ATP, they competitively bind to ATP when they coexist. As a result, the ATP modulation effect was not as high as predicted since a part of the ATP molecules were being consumed by the clamp loader. The interaction between MutL and the δ′ subunit was an exception in that it was not modulated by ATP (data not shown).

3.4. The 293 N-terminal amino acids and 59 C-terminal amino acids of MutL are required for interaction with the subunits δ and δ′

A series of N- and C-terminal deletions of MutL were generated by gene manipulation in an effort to localize the interaction domain to smaller regions of the 2 proteins (Fig. 5A). As shown in Fig. 6, compared with wild-type MutL, the interactions between the N-terminal deletion mutants MutLΔ294N, MutLΔ397N, and MutLΔ438N and subunits δ and δ′ were negligible. Moreover, most of the interactions of the C-terminal deletion mutants MutLΔ283C and MutLΔ59C were eliminated. This result indicated that both the N- and C-termini of MutL are essential for the interactions with the δ and δ′ subunits, and the MutL N-termini appears to have higher affinity for δ and δ′. Strangely, all deletions showed no
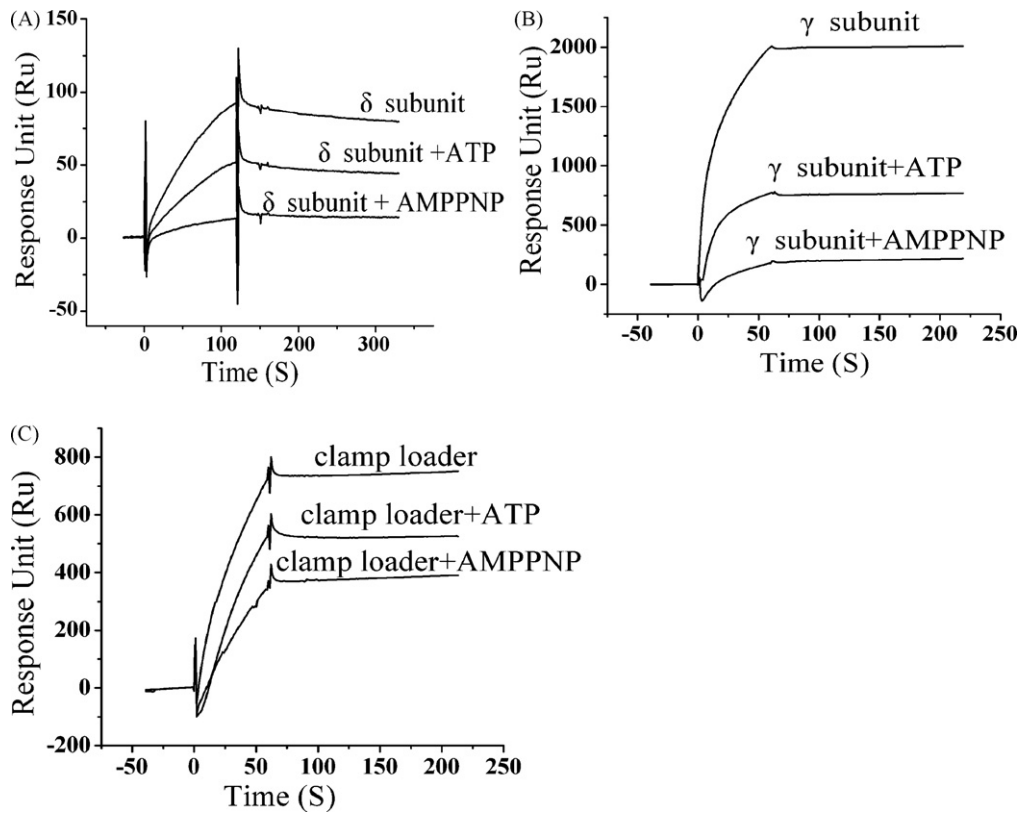
Fig. 4. ATP modulation on the interactions of MutL with the subunits δ, γ and clamp loader. Immobilized MutL sensors chips were used in this experiment. (A) Responses of δ subunit with or without ATP or AMPPNP; (B) responses of γ subunit with or without ATP or AMPPNP; (C) responses of clamp loader with or without ATP or AMPPNP. All the mixtures were incubated for 5 min at 37 °C before injection. Concentrations of the components were 200 nM for δ subunit, γ subunit and clamp loader, 1 mM for ATP and AMPPNP, respectively. This experiment had been repeated or at least 3 times.

apparent effect on the interactions between MutL and the γ subunit.

4. Discussion

In this study, several interactions between the MMR key protein MutL and the clamp loader subunits were experimentally observed, and most of the interactions were noted to be modulated by ATP. As a number of technologies (far western, SPR, and deletion analysis) were applied for this study, false positives were largely precluded and reliable results could thus be obtained. Based on these new findings and previous reports, we were able to determine the network of interactions between these 2 systems (Fig. 7).

The role of the MutL protein, characterized as the master regulator of MMR, remains to be completely defined on a mechanistic level. The solved crystal structure of the N-terminal domain of MutL [27,28] demonstrated an ATP binding/hydrolysis fold common to the GHKL group (gyrase/Hsp90/histidine-

kinase/MutL) of ATP-hydrolyzing enzymes [29]. We conclude from previous results and the data in this study that MutL has at least 3 functions. First, it binds to the MutS-DNA-ATP complex and activates the endonuclease activity of MutH [30]. Second, it helps to load UvrD onto DNA in the presence of ATP, which unwinds dsDNA during the MMR process [29]. Third, the strong binding of MutL to the δ, δ', and γ subunits and the clamp loader suggests that MutL is involved in the recruitment of the clamp loader onto the single-stranded gap after the incision of the unmethylated strand. Interestingly, a recent study of MMR in a bacterial cell also showed that approximately half of the MutS and MutL foci colocalized with the DNA polymerase foci [31].

Although it is known that DNA polymerase III is required in MMR, the mechanistic basis of this requirement is entirely unclear. The demonstration of specific interactions between MutL and the polymerase III clamp loader subunits provides some insights into this matter. When MutL interacted with the subunits δ and γ and the
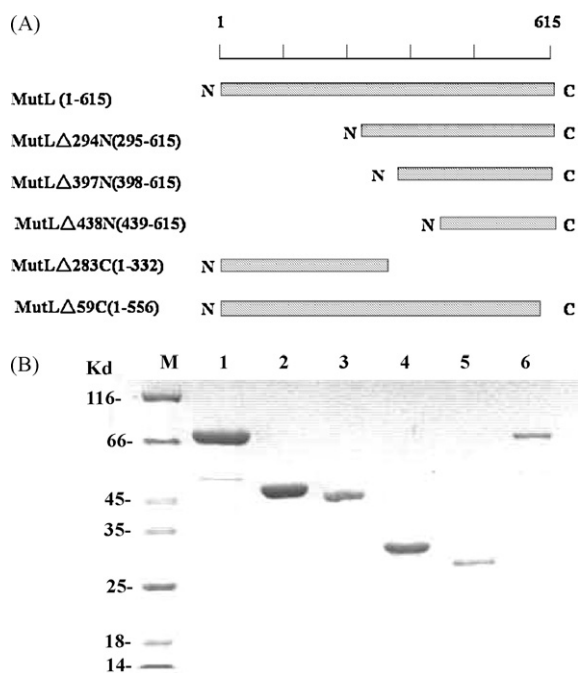
Fig. 5. Construction and purification of *mutL* mutants. (A) All *mutL* deletions were constructed in pQE30-*sbp* and were purified by NTA-Ni resin, (B) SDS-PAGE analysis of different purified MutL-SBP deletion mutant, sample loading size was 10 µl. Lane 1, MutL; lane2, MutLΔ283C; lane 3, MutLΔ294N; lane 4, MutLΔ397N; lane 5, MutLΔ438N; lane 6, MutLΔ59C.

clamp loader in the presence of ATP or AMPPNP, the RU value changed significantly and the effects of ATP and AMPPNP could be distinguished. This implies that ATP was hydrolyzed during the interaction and thus had less effect. The integrity of the MutL homologue ATP center is required for function in MMR, and ATP appears to extensively modulate the newly identified interactions. Since no ATPase site was found in the δ subunit [18], the possible explanation of the above phenomenon is that ATP was hydrolyzed by MutL. MutL interacts with and modulates the activities of MutS, MutH, and UvrD. The purified protein catalyzes a slow ATP hydrolysis reaction that is essential for its function in MMR. However, the role of the ATP hydrolysis reaction is not understood. Recent research also demonstrated that ATP hydrolysis by MutL is an essential step in the MMR pathway subsequent to the loading of UvrD [32]. We therefore conclude that ATP is hydrolyzed by MutL during the recruitment step; this provides useful indications for better understanding the role of the ATP hydrolyzed by MutL.

Fig. 7 indicates that MutL appears to be the core element that interacts with many other proteins. The homologues of this "sticky" protein in humans are involved in many biological processes such as intracellular transport, cell signaling, cell morphology, recombination, ubiquitylation [33]. The structure of the clamp loader may be attributed to the interactions between MutL and the 3 subunits as a whole. The crystal structure of the clamp loader yielded significant indications regarding the structural similarities between the δ subunit and the δ′ and γ subunits [34,35]. In addition, the subunits δ, δ′, and γ are all structurally related to the AAA+ ATPases [18]. Further, all the 3 subunits in the same complex can interact with MutL, contributing to highly efficient recruitment during the excision step that would result in the effective re synthesis of a long gap produced after excision.

Deletion analysis demonstrated that both the N- and C-termini of MutL were required to maintain the interactions with subunits δ and δ′. Similar domain-specific interactions were observed in the interactions between MutL and UvrD and between MutL and MutH. Both the N- and C-terminal domains of MutL can interact with UvrD or MutH [36]. We were unable to localize the region of MutL responsible for binding the γ subunit because the interactions were evidenced in all deletion mutants. One explanation is that the γ subunit adopts a very different conformation due to differences in the interdomain arrangements [18].

The β clamp, one of the DNA polymerase III subunits, is another important component in the network as shown in Fig. 7. The β clamp communicates directly with multiple proteins to promote DNA replication and DNA repair [37,38]. The clamp loader is required to rapidly open the β ring and then place it around the DNA in an ATP dependent reaction. The clamp loader leaves, permitting other enzymes, such as polymerase III, to target the clamp for processive synthesis. Although MutL was observed to interact with the clamp loader subunits δ, δ′, and γ, some questions continue to remain unanswered. For example, a current model of MMR in *E. coli* posits a set of carefully orchestrated steps [29]; however, the mechanism underlying the interactions that occur when MutL recruits the clamp loader subunits is still a matter of debate.

In conclusion, this study reveals the close relationship between the *E. coli* MMR proteins and the DNA polymerase III subunits and the ATP modulation of these interactions. The interaction network formulated based on established information and the new findings provide strong evidence to support the notion that DNA replication and MMR are highly associated with each other. Details regarding the specific biological functions associated with these interactions are worth investigating. In addition, the discovery of the physical interactions
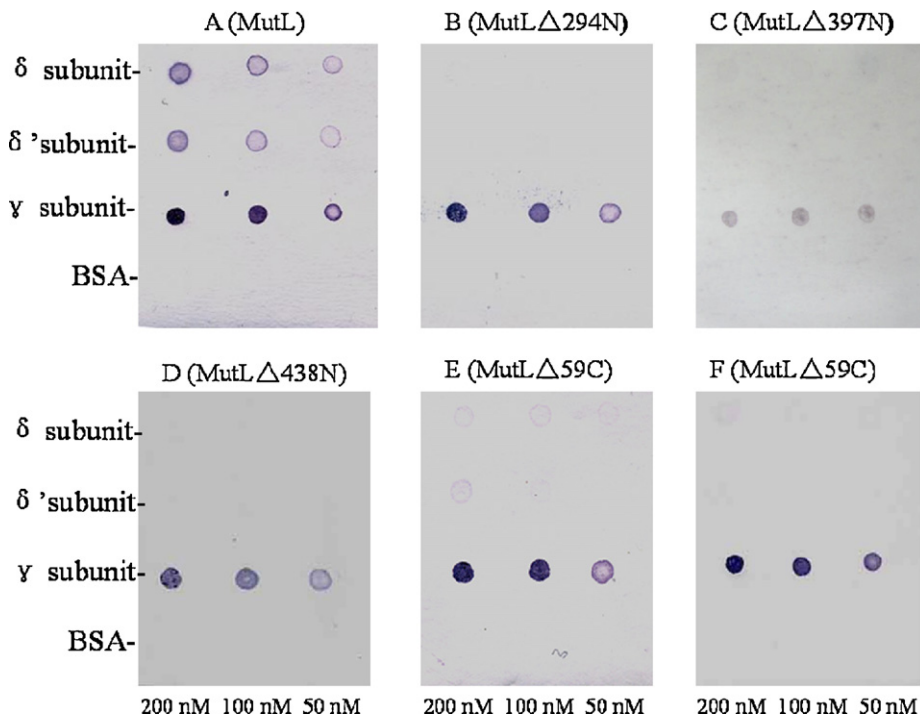
Fig. 6. Far western analysis of effect of MutL N/C terminal deletions on the interactions between MutL and clamp loader subunits δ, δ′ and γ. The indicated amount of subunits δ, δ′, γ and BSA (200 nM to 50 nM, from left to right) were applied to nitrocellulose membrane, details has been described in Section 2.
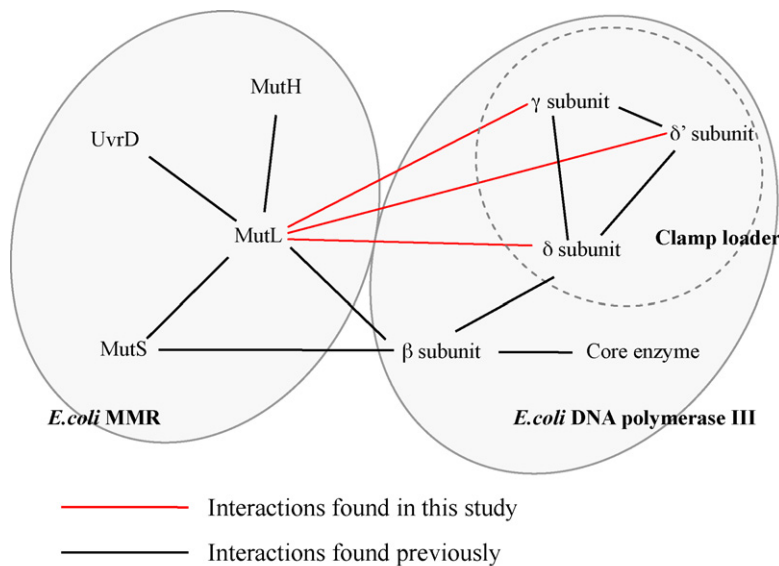

Fig. 7. Interaction network between *E. coli* MMR system and *E. coli* polymerase III subunits. The known interactions are black lines and the newly found interactions are red lines.

between MutL and the clamp loader subunits may lead to advances in our understanding of MMR in the eukaryotic system.

Acknowledgments

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