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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.

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 instability 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 *Escherchia 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™ 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 LEHHPQGQREP) coding sequence together with a linker peptide $(Gly_3\text{-}Ser_3\text{-}Gly_3)_3$ 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 (λ = 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