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Brief communication

Characterization of the defects in the ATP lid of *E. coli* MutL that cause transient hypermutability

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ABSTRACT

Mutator strains spontaneously arise in bacterial populations under stress in an attempt to increase evolutionary adaptation. Inactivation of the ubiquitous DNA mismatch repair pathway, whose normal function is to correct replication errors and hence increase replication fidelity, is often the cause of the mutator phenotype. One of the essential genes in this pathway, *mutL*, includes a short tandem repeat that is prone to polymerase slippage during replication. While extensive work has established that this repetitive sequence is a genuine genetic switch, the mechanism of MutL inactivation remains unclear. This short tandem repeat is translated into a LALALA motif that resides near the ATPase active site of MutL. Therefore, changes in the length of this motif are presumed to alter the ATPase activity of MutL. We have engineered variants of *Escherichia coli* MutL with shorter/longer LALALA motifs and characterized their ATPase and DNA binding functions. We have found that the deletion or insertion of a single LA repeat did not compromise the structural integrity of the protein, nor did it affect MutS- or DNA-binding activity. However, it severely compromised ATP binding and, consequently, engagement of the N-terminal domains; both essential activities for proper DNA mismatch repair. These results are discussed in the context of the structure of MutL.

1. Introduction

Bacteria have evolved toward a low DNA mutation frequency in stable environments because mutations often have deleterious effects [1,2]. However, bacterial populations in a new environment are confronted with the need to adapt. Since asexual populations rely on mutagenesis to increase genetic variation, bacteria tend to have hotspots in antimutator genes, such as DNA repair genes [3]. To compensate for the inevitable deleterious impact of maintaining a high mutation rate, bacterial mutator phenotypes are often transient so the population can spontaneously revert back to a low mutation frequency [2].

Defects in the conserved post-replicative DNA mismatch repair (MMR) pathway are often the cause of reversible hypermutability in bacteria. MMR increases fidelity of DNA replication by repairing mismatched bases and small insertion/deletion loops introduced during DNA synthesis. Three key proteins initiate the repair response (reviewed in [4,5]). MutS recognizes and binds to a mismatch and recruits the molecular matchmaker MutL in an ATP-dependent manner [6]. The ATP-bound form of MutL can then activate the latent endonuclease MutH which, in turn, nicks the newly synthesized strand at the nearest hemimethylated GATC site [7,8]. The nick marks the nascent strand for repair by providing an entry point for the UvrD helicase and exonucleases to unwind and degrade the erroneous strand, thereby providing the replication machinery with a second chance to re-synthesize the strand correctly [5]. While MutS and MutL are evolutionary conserved, MutH is only present in a subset of gamma-proteobacteria including *Escherichia coli* (*E. coli*). In organisms lacking MutH, MutL harbors a latent endonuclease activity that uses the gap left by the removal of misincorporated ribonucleotides as the strand discrimination signal [9,10] and provides entry sites for downstream MMR factors [11–16].

Several independent studies have linked bacterial hypermutability to mutations in a conserved short tandem repeat at the beginning of the *mutL* gene [17–21]. Sequencing of the *E. coli mutL* gene from hypermutator strains revealed either a deletion or an insertion in a triple hexanucleotide tandem repeat

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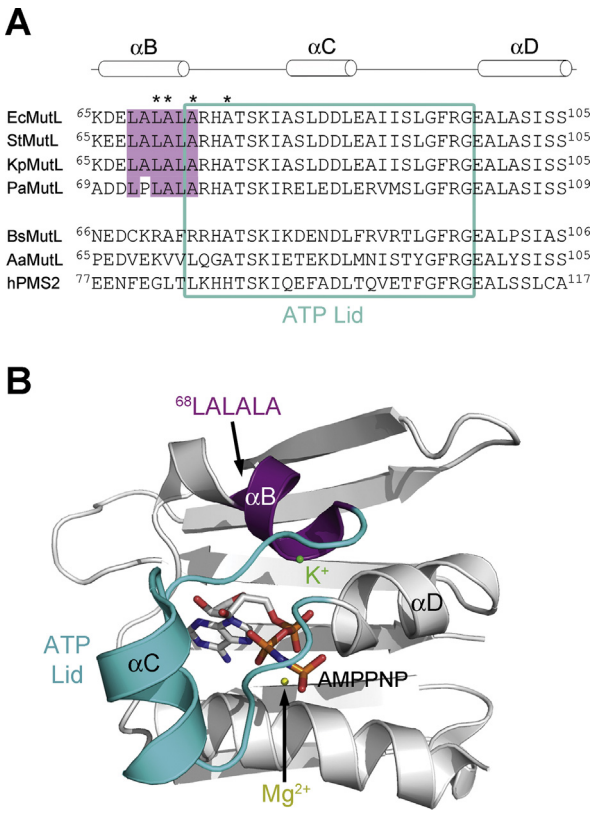

Fig. 1. The LALALA motif is part of the ATP binding pocket. (A) Sequence alignment of the MutL ATP lid from organisms in the gamma-proteobacteria class (*E. coli* K12 (Ec), *Salmonella typhimurium* LT7 (St), *Klebsiella pneumoniae* (Kp), and *Pseudomonas aeruginosa* (Pa)) and from organisms outside this class (*Bacillus subtilis* (Bs), *Aquifex aeolicus* (Aa), and human MutL homologue PMS2 (hPMS2)). The secondary structure corresponds to the *E. coli* MutL crystal structure (PDB ID 1B63). The lid region is indicated by a blue box and the LALALA motif is highlighted in purple. *E. coli* MutL residues involved in chelating the monovalent metal ion are indicated by black asterisks. (B) Ribbon diagram of the *E. coli* MutL nucleotide binding pocket (PDB ID 1NHI). The LALALA motif is shown in purple and the ATP lid is in blue. The AMPPNP molecule is shown as color-coded sticks and the Mg^{2+} and K^{+} ions are shown as yellow and green spheres, respectively. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

(5′CTGGCGCTGGCGCTGGCG3′) between nucleotides 213–230 [18]. Alterations to this repeat are likely the result of strand slippage, making the mutator phenotype reversible [18]. At the protein level, this repeat results in a LALALA motif that resides in the N-terminal domain of MutL and it is strictly conserved among MutL homologues found in gamma-proteobacteria encoding a *mutH* gene, loosely conserved in gamma-proteobacteria that do not encode a *mutH* homologue (Fig. 1A), and absent outside the proteobacteria phylum [20].

MutL homologues consist of two structurally conserved domains connected by a flexible linker [4]. The N-terminal domain supports DNA-binding and ATPase activity, while the C-terminal domain harbors a constitutive dimerization interface [22–24]. The N-terminal domain of MutL belongs to the GHKL superfamily of phosphoryl transferases. This family is characterized by the presence of four conserved motifs defining a Bergerat fold [25–27]. In the case of MutL, the loops surrounding the ATP binding site are disordered in the absence of nucleotide, but they become ordered upon ATP binding [22,23]. The LALALA motif precedes one of these loops, the ATP lid that on its ordered–closed–conformation covers the nucleotide binding site (Fig. 1B). The closed conformation of the lid is stabilized by the coordination of a monovalent cation [28]. A second metal binding site harbors the catalytic magnesium ion [22]. Similar to other phosphoryl transferases, both metal ions are required for optimal ATP binding and, in turn, for proper mismatch repair [28,29]. ATP binding triggers the association of the N-terminal domains resulting in a closed conformation [12,22,24,30,31]. Upon ATP hydrolysis, the N-terminal domains dissociate to revert back to an open conformation. Cycling between the open and closed conformation regulates downstream MMR events. In particular, ATP binding has been shown to have a stimulatory effect on MutL endonuclease activity in organisms lacking MutH [11,13–16].

The role of this short tandem repeat as a genetic switch is well established, however the mechanistic defects associated with variations in the LALALA motif are unknown. In this work, we have produced MutL variants with a deletion (MutL-2LA) or an insertion (MutL-4LA) in the LALALA motif and characterized their ability to bind MutS, DNA, as well as to bind and hydrolyze ATP, to reveal the functional defects that cause the acquisition of the strong mutator phenotype.

2. Materials and methods

2.1. Cloning MutL variants

E. coli MutL (pTX418, residues 1–615) was a kind gift from Dr. Wei Yang (LMB, NIDDK). *E. coli* MutL-2LA (pAG8529, ^{66}DELALALARH^{75}–^{66}DELALARH^{73}) and MutL-4LA (pAG8705, ^{66}DELALALARH^{75}–^{66}DELALALALARH^{77}) were generated by overlap PCR, cloned into the pET15b expression vector (Novagen), and verified by DNA sequencing (MOBIX, McMaster University).

2.2. Protein expression and purification

MutL, MutL-2LA and MutL-4LA were produced in *E. coli* BL21(DE3) cells (Invitrogen), protein expression was induced with 1 mM IPTG for 3 h at 37 °C (MutL and MutL-4LA) or 5 h at 25 °C (MutL-2LA). Cells were harvested by centrifugation, the cell pellets resuspended in buffer A (20 mM Tris pH 8.0, 1.4 mM β-mercaptoethanol, and 0.5 M NaCl) and lysed by sonication. Lysates were clarified by centrifugation at 39,000 × *g*, loaded onto a HiTrap nickel-chelating column (GE Healthcare) equilibrated with buffer A, and eluted with 0.3 M imidazole. The sample was further purified using a Q-sepharose column (GE Healthcare) equilibrated using buffer B (20 mM Tris pH 8.5, 90 mM KCl, 5 mM DTT, 1 mM EDTA, and 10% glycerol) and MutL was eluted with a linear salt gradient to 240 mM KCl. MutL was loaded onto a Superdex-200 (GE Healthcare) equilibrated with storage buffer (20 mM Tris pH 8.5, 5 mM DTT, 1 mM EDTA, 150 mM KCl, and 10% glycerol).

2.3. Dynamic light scattering

Dynamic light scattering was performed using a Zetasizer Nano S (Malvern Instruments). All measurements were taken using a 12 μL quartz cell (ZEN2112) at 4 °C. Size distribution of the samples was calculated based on the correlation function provided by the Zetasizer Nano S software.

2.4. DNA binding assay

DNA binding activity was evaluated using supercoiled DNA as described previously [16], with minor changes. MutL variants (final concentration, 833 nM) were incubated with supercoiled pUC19 plasmid DNA (5 nM) in DNA binding buffer to a final reaction volume of 15 μL. Reaction mixtures were then incubated for 90 min at 37 °C and resolved on 1% Tris–Acetate–EDTA agarose gels. Binding to linear DNA was assessed using 68 base-pair substrates as described earlier [24]. In brief, single- or double-stranded DNA