

Endonuclease Activity of MutL Protein of the *Rhodobacter sphaeroides* Mismatch Repair System

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Abstract—We have purified the MutL protein from *Rhodobacter sphaeroides* mismatch repair system (rsMutL) for the first time. rsMutL demonstrated endonuclease activity *in vitro*, as predicted by bioinformatics analysis. Based on the alignment of 1483 sequences of bacterial MutL homologs with presumed endonuclease activity, conserved functional motifs and amino acid residues in the rsMutL sequence were identified: five motifs comprising the catalytic site responsible for DNA cleavage were found in the C-terminal domain; seven conserved motifs involved in ATP binding and hydrolysis and specific to the GHKL family of ATPases were found in the N-terminal domain. rsMutL demonstrated the highest activity in the presence of Mn²⁺. The extent of plasmid DNA hydrolysis declined in the row Mn²⁺ > Co²⁺ > Mg²⁺ > Cd²⁺; Ni²⁺ and Ca²⁺ did not activate rsMutL. Divalent zinc ions inhibited rsMutL endonuclease activity in the presence of Mn²⁺ excess. ATP also suppressed plasmid DNA hydrolysis by rsMutL. Analysis of amino acid sequences and biochemical properties of five studied bacterial MutL homologs with endonuclease activity revealed that rsMutL resembles the MutL proteins from *Neisseria gonorrhoeae* and *Pseudomonas aeruginosa*.

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All living organisms, from bacteria to humans, possess a system for repair of noncanonical nucleotide pairs (mismatches) and small loops, which was named the DNA mismatch repair (MMR) system [1-3]. Impairments in MMR at any stage of DNA repair cause accumulation of mutations, resulting in the development of various types of tumors [4, 5]. Because of the essential

role of MMR in functioning of any living organism, studies of MMR are of extreme importance.

MMR corrects DNA damage by excising an extended single-stranded fragment of the newly synthesized DNA and then filling the resulting gap. The signal for the MMR is hydrolysis of the phosphodiester bond in one of the DNA strands [6-8]. In cells of γ -proteobacteria, including *Escherichia coli*, this break is introduced by the MutH protein that recognizes the monomethylated 5'-Gm⁶ATC-3'/3'-CTAG↓-5' sequence (m⁶A is 6-methyl-2' deoxyadenosine; ↓ is the site of hydrolysis). The catalytic activity of MutH is stimulated by the complex of the MutS and MutL proteins [9]. MutH homologs are absent in eukaryotes and other types of bacteria that do not use methylation as a marker of the nascent DNA strand.

Abbreviations: a.a., amino acid; bp, base pair; DTT, 1,4-dithiothreitol; ecMutL, MutL from *Escherichia coli*; GHKL, Gyrase, Hsp90, Histidine Kinase, MutL family of ATPases; IPTG, isopropyl β -D-1-thiogalactopyranoside; LB, lysogeny broth; MMR, mismatch repair system; PMSF, phenylmethylsulfonyl fluoride; rsMutL, MutL from *Rhodobacter sphaeroides*.

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According to the existing hypothesis, the key role in the strand discrimination in these organisms belongs to the DNA polymerase III β -subunit that determines the strand polarity and directly interacts with the MMR proteins [1]. However, this hypothesis is still debated.

MutL is a weak dimeric ATPase from the GHKL family [10]. For a long time, it had been assumed that the major role of MutL is coordination of protein–protein interactions during recognition and removal of one of the DNA strands; hence, MutL was classified as a molecular mediator [11]. However, discovery of the endonuclease activity of eukaryotic MutL α and then of bacterial MutL homologs in organisms that lack the MutH gene led to the suggestion of a key role of MutL in repair initiation [12, 13]. It was found that in these organisms, MutL has a conserved endonuclease motif that is supposedly required for the introduction of a single break in the nascent DNA strand [14]. The mechanism of this process is virtually unexplored; bacterial MMR systems with the endonuclease activity of MutL are considerably less studied than the MMR in *E. coli* and humans. At the same time, the MMR system of bacteria is particularly interesting because of high functional and species diversity of prokaryotes.

MutL proteins possessing the endonuclease motifs have been purified from only five bacterial species: *Pseudomonas aeruginosa*, *Neisseria gonorrhoeae*, *Thermus thermophilus*, *Aquifex aeolicus*, and *Bacillus subtilis* [15–19]. All of them are homodimers, which are simpler and easier to study than their heterodimer analogs in eukaryotes. Therefore, the data obtained for bacterial MutL proteins can be used for the reconstruction of the major stages of the MMR mechanism at the molecular level.

In this work, we developed a method for MutL (rsMutL) purification from *Rhodobacter sphaeroides*. Interest in this rod-like Gram-negative purple nonsulfur photoheterotrophic α -proteobacteria is due to the variability of its metabolic and life cycles, photosynthetic activity, and ability to survive under diverse environmental conditions [20, 21]. We analyzed the primary structure of rsMutL, predicted its secondary structure, and identified conserved amino acid residues and motifs by comparing amino acid sequences of many rsMutL bacterial analogs with supposed endonuclease activity. We also demonstrated that rsMutL hydrolyzes DNA, and its activity is affected by nonprotein factors such as divalent metal cations and ATP. We compared our results with the data of a few studies of the mechanism of action of bacterial MutL proteins with endonuclease activity.

MATERIALS AND METHODS

Bioinformatics analysis of the rsMutL sequence. The sequence of MutL protein from *R. sphaeroides* strain 2.4.1 was obtained from the UniProt database (accession number Q3IYV3). The sequence of MutL from *E. coli* strain

K12 (ecMutL; UniProt accession number P06722) was also used for searching for MutL homologs. We searched 4379 representative bacterial and archaeal genomes (representative microbial genomes sample in the Refseq database, NCBI) for rsMutL and ecMutL homologs using the TBLASN program (e-value, 0.001; word length, 3).

The sequences were aligned with the Muscle program using default settings. All rsMutL and ecMutL homologs were divided into three groups based on the presence of the endonuclease motif **XII** (Table 1). To distinguish groups among all full-size MutL homologs, we chose the rsMutL fragment 449–462 (14 a.a.) that included motif **XII**. All the obtained sequence fragments were clustered according to the percentage of functionally similar amino acid residues using the Protdist program from the PHYLIP software package. The largest cluster included fragments from proteins with proven endonuclease activity. Proteins of this cluster were assigned to group 1 with presumable endonuclease activity. Proteins that lacked sequences reliably similar to motif **XII** and, therefore, had no endonuclease activity, were assigned to group 2. Group 3 included proteins whose fragments had low but significant similarity to motif **XII**. In most of these proteins, at least one of the essential residues in motif **XII** [14] was substituted by a functionally different amino acid.

Then we aligned sequences of rsMutL and ecMutL homologs from groups 1 and 2, respectively, except sequences that lacked the *N*-terminal fragments (including motif **I**, at least partially) or *C*-terminal fragments (including motif **XVI**, at least partially) (Fig. 1). Only one sequence from each group with 95% identity was retained in the final alignment. Conserved residues were identified with a specially written program.

Supplemental materials can be found on the website <http://mouse.belozersky.msu.ru/NPIDB/MutL/> and include:

- 1) Alignment 1 of 1483 amino acid sequences of proteins with predicted endonuclease activity and ecMutL sequence (for comparison) in the FASTA format. A subgroup of 93 sequences lacking endonuclease motifs **XIV** and **XV** occupies the lowest rows in the alignment.
- 2) Alignment 2 of 309 amino acid sequences of proteins presumably lacking endonuclease activity and rsMutL sequence (for comparison) in the FASTA format.
- 3) The names and taxonomic groups of bacteria and archaea whose sequences were used for alignment.

Construction of plasmids containing the rsMutL gene. The plasmid encoding rsMutL with an additional *N*-terminal sequence (36 a.a.) including the His₆ tag (His₆-linker-rsMutL) was obtained as described in [16]. The rsMutL gene was amplified by PCR using *R. sphaeroides* (strain 2.4.1.) genomic DNA as a template. DNA was isolated from *R. sphaeroides* cells by the alkaline lysis method [27]. The primers 5'-AAGGGGC-CATATGGGAAGCCGGTGGCGGAG-3' and 5'-AA-

Table 1. Conserved motifs in bacterial MutL proteins and their functions

Motif, domain	Function	Consensus sequence [22]*; sequence in rsMutL
I, N	unstructured loop involved in dimerization of N-domains; affects ATP binding [23]	XXXIXILXDXLXNX IAAGEVV 12 RPVIRQLDEAAINR IAAGEVV 32
II, N	directly interacts with bound ATP; Glu from KEL activates water molecule for ATP hydrolysis; Asp binds Mg ²⁺ [23, 24]	VVKELVENALDAGA 38 AVKELVENALDAGA 51
III, N	all residues are directly involved in ATP binding [23]	DNGXGM 70 DDGCGM 75
IV, N	unstructured loop involved in the interface formation for the interaction with other proteins after ATP binding and protein structure stabilization [23]	RHATSKI 86 RHATSKI 92
V, N	presumably forms hydrogen bonds with the ATP γ -phosphate group [23]	TLGFRGEALXS 102 SFGFRGEALPS 112
VI, N	responsible for the interaction with MutS [25]	[K/H/R/E]X _n [K/H/R/E] [I/L]X[I/L/V] 118 RLTITSRVAEAEGAQ --- I --- A --- V 135
VII, N	ATP-binding motif	GT 151 GT 152
VIII, N	interacts with DNA polymerase III β -subunit (β -clamp) [26]	LEFNTPARRKFLK 159 LEFFATPARLKFLR 171
IX, N	ATP-binding motif; Asn stabilizes ATP γ -phosphate group [23]	NGR 273 NGR 275
X, N	unstructured loop; directly interacts with ATP [23]	VDVNVHPXKXEVR FXX 313 VDVNVHPAKAEVR FRE 328
XI, C	indirectly contributes to stabilization of the endonuclease active site [19]	GQ 432 AQ 433
XII, C	responsible for metal ion binding (Glu) and MutL endonuclease activity [19]	DQHAAHERILYE 451 DQHAAHERLVYE 462
XIII, C	Pro is presumably responsible for correct orientation of the motif required for the interaction with the β -clamp [26]	QXLLIP 476 QALLIP 481
XIV, C	endonuclease motif	ACK 562 ACH 564
XV, C	Cys and His are involved in the formation of the Zn ²⁺ -binding site [19]	CPHGRP 594 CNHGRP 599
XVI, C	endonuclease motif	FXR 613 FGR 615

* The consensus motifs were identified in [22] by alignment of 208 amino acid sequences of bacterial MutL proteins; endonuclease motifs were identified by alignment of 192 sequences. Bold letters in gray boxes, identical residues; bold letters, conserved (over 90%) residues. Amino acid residues comprising the consensus motifs in MutL are underlined.

GGGGAAGACGAGATCTTCATCGCCGGCCGAA-GAGCC-3' contained the recognition sites of BglII and NdeI restriction endonucleases, respectively. The resulting PCR product was treated with BglII/NdeI and cloned into the pET15b plasmid vector that contained the sequence encoding an additional 36-a.a. fragment including the His₆-tag.

Plasmid constructs for expression of rsMutL tagged with His₆ directly at the N- or C-ends (His₆-rsMutL and rsMutL-His₆, respectively) were obtained using the pVax1-MutL plasmid (derivative of the pVax1 plasmid) that contained the rsMutL gene optimized for expression in *E. coli* (Evrogen, Russia). DNA sequences coding for His₆-rsMutL and rsMutL-His₆ were cloned into the

pET-45b(+) (Novagen, Germany) and pQE13 (Qiagen, Germany) vectors by the BamHI/AgeI or BamHI/KpnII sites, respectively. Cloning was verified by restriction analysis and sequencing.

Purification of rsMutL protein. *Escherichia coli* BL21(DE3) cells were transformed with the His₆-linker-rsMutL and His₆-rsMutL plasmids, and M15(pREP4) cells were transformed with the rsMutL-His₆ by the heat-shock method. The cells were grown in 3 liters of LB medium containing 100 µg/ml ampicillin on a shaker at 37°C to the optical density $A_{600} = 0.5$. Protein expression was induced by adding 0.1 mM isopropyl β-D-1-thiogalactopyranoside (IPTG), and the cells were incubated for another 16–18 h at 18°C. The cells were collected by centrifugation; the pellet was frozen and stored at –20°C. Protein expression was monitored by withdrawing aliquots before and after induction and analyzing them by electrophoresis in 8% SDS-polyacrylamide gel. The gels were stained with PageBlue™ solution (Thermo Fisher Scientific, USA) containing Coomassie Brilliant Blue G-250.

Frozen cells were thawed on ice for 30 min, resuspended in buffer A (20 mM Tris-HCl, pH 7.9, 1 M NaCl, 1 mM 1,4-dithiothreitol (DTT), 5% glycerol (v/v), 0.05% Tween 20 (v/v), and 1 mM phenylmethylsulfonyl fluoride (PMSF)) containing 5 mM imidazole, and disintegrated by sonication. The obtained cell lysate was centrifuged at 18,000 rpm (JA-10 rotor, fixed angle; Beckman, USA) for 30 min at 4°C to remove the debris. The supernatant was added to 1 ml of Ni-NTA-agarose (Thermo Fisher Scientific) and incubated for 1 h at 4°C with gentle mixing. The sorbent with the bound protein was separated by centrifugation at 800 rpm (F-35-6-30 rotor, fixed angle; Eppendorf, Germany) for 5 min at 4°C. The supernatant was removed except the last 10 ml that were used for the resuspension of the sorbent and its transfer to a 15-ml column at 4°C. After sedimentation of the sorbent, the liquid was allowed to drain, and the sorbent was washed with 15 ml of buffer A containing 60 mM imidazole to remove unbound proteins. The rsMutL was eluted from the column with 500 µl of buffer A containing 500 mM imidazole. The collected fraction was loaded on the column again, and the whole procedure was repeated 2–3 times. Final elution was performed with 500 µl of fresh buffer; DTT was added to the eluate to the final concentration of 10 mM.

rsMutL was further purified by size-exclusion chromatography on a 10/300-mm ENrich SEC 650 column (Bio-Rad, USA). The column was equilibrated with 10 mM HEPES-KOH buffer (pH 8.0) containing 300 mM KCl, 1 mM DTT, 5% glycerol (v/v), and 0.05% Tween 20 (v/v); the same buffer was also used as an eluent. The protein was loaded on the column in a volume of 1 ml and eluted at a rate of 1 ml/min. Elution was monitored at 280 nm; protein-containing fraction were collected and analyzed by electrophoresis in 8% SDS-poly-

acrylamide gel. Protein concentration in the fractions was determined spectrophotometrically with a NanoDrop ND-1000 spectrophotometer (PecLab, USA). The collected fractions were concentrated using Amicon Ultra centrifuge concentrators (Merck, Germany) with 10-kDa cut-off membrane. After concentration, protein fractions were aliquoted (5–10 µl), flash-frozen in liquid nitrogen, and thawed immediately before use.

Hydrolysis of plasmid DNA by rsMutL. The pUC-MMR plasmid (10–20 nM) used earlier for studying MMR in *E. coli* [28] was incubated with rsMutL in 10 mM HEPES-KOH (pH 8.0) containing 100 mM KCl and 5 mM divalent metal salt at 37°C for 0–90 min. The reaction was terminated by adding 50 mM EDTA and 10 U of proteinase K with subsequent incubation of the mixture for 30 min at 37°C. To develop the optimal reaction conditions, the rsMutL concentration was varied from 0.1 to 2 µM. The endonuclease activity of rsMutL was assayed in the presence of MgCl₂, MnCl₂, CaCl₂, CoCl₂, NiCl₂, CdCl₂, or ZnCl₂ (5 mM). The ATP concentration varied from 0 to 5 mM. The reaction products were analyzed by electrophoresis in 1% agarose gel containing 0.5 µg/ml bromide in TAE buffer (40 mM Tris-acetate, pH 7.5, 1 mM EDTA). The gels were photographed, and the images were processed with the Image Lab software (Bio-Rad). In the control experiments, the pUC-MMR plasmid (20 nM) was incubated with BamHI or nicking endonuclease Bpu10I for 60 min at 37°C in 10 µl of 10 mM Tris-HCl (pH 8.0) containing 5 mM MgCl₂, 100 mM KCl, 0.02% Triton X-100, and 0.1 mg/ml BSA (for BamHI) or 10 mM Tris-HCl (pH 8.5) containing 10 mM MgCl₂, 100 mM KCl, and 0.1 mg/ml BSA (for Bpu10I).

RESULTS AND DISCUSSION

Characterization of the primary and secondary structures of rsMutL. The sequence of MutL from *R. sphaeroides* strain 2.4.1 was obtained from the UniProt database (Q3IYV3). rsMutL consists of 616 a.a. and has a molecular mass of 65.8 kDa. Its isoelectric point is 6.51 as determined with the Protein Calculator v. 3.4 software.

Proteins of the MutL family are composed of *N*- and *C*-terminal domains connected by a variable linker [1]. According to the Pfam database, the *N*- and *C*-terminal domains of rsMutL include a.a. 1–343 and 427–616, respectively. It was found that MutL sequence contains 16 conserved motifs [22]. To identify the same motifs in rsMutL, we aligned the rsMutL sequence with nine sequences of other MutL proteins. For some of these proteins, positions of the conserved motifs have been already determined (Fig. 1). We found that rsMutL contained all 16 motifs (Fig. 1 and Table 1). All of them (except VI) coincided with the consensus sequences of the earlier identified motifs [22]. Seven conserved motifs (I, II,

III, V, VII, IX, and X) presumably form the ATP-binding site in rsMutL (Table 1). The sequences corresponding to motifs **VIII** (*N*-terminal domain) and **XIII** (*C*-terminal domain) might be involved in the interaction with the β -clamp.

The motif **VI** localized in the *N*-terminal domain is essential for the MutS protein binding. It is not conserved enough to predict which residues in this motif are involved in the interaction with MutS. Endonuclease activity was experimentally demonstrated for MutL proteins from bacteria belonging to five different classes: β -proteobacteria (*N. gonorrhoeae*), Bacilli (*B. subtilis*), γ -proteobacteria (*P. aeruginosa*), Deinococci (*T. thermophilus*), and Aquificae (*A. aeolicus*). The sequences of MutL proteins from these bacterial species were included in the alignment in Fig. 1. All of them possess a full set of endonuclease motifs (**XI, XII, XIV-XVI**) that form the catalytic site for DNA hydrolysis (Table 1). These motifs are absent in MutL proteins lacking endonuclease activity (*E. coli* MutL protein and human MLH1 in the alignment).

Previous analysis of 208 MutL sequences revealed several conserved motifs in the protein primary structure [22]. To estimate with higher reliability how conserved and, therefore, functionally significant these motifs are, we analyzed a wider range of bacterial and archaeal MutL homologs. Initially, this sample included 2544 sequences: 1483 of them we predicted to have the endonuclease activity (alignment 1); 309 sequences presumably lacked this activity (alignment 2). The sequences were selected based on the presence/absence of the endonuclease motif **XII**, which has the highest informational content among all endonuclease motifs.

We compared the number of conserved or functionally conserved (by 90% or higher) positions in the MutL sequences. The *N*-terminal domains of MutL proteins from groups 1 and 2 contained 71 and 82% of conserved residues, respectively; 67% of these residues overlapped. The content of conserved residues in the *C*-terminal domains of proteins from groups 1 and 2 comprised 50 and 34%, respectively; 24% of these residues overlapped. The higher content of conserved residues in the *C*-terminal domain in proteins with presumed endonuclease activity confirmed our expectations and indirectly proved the validity of MutL protein classification into groups – it is known that enzyme active sites, residues in close vicinity to the active sites, and ligand- and ion-binding sites are generally more conserved than the sequence fragments at the protein globule surface, including those involved in protein–protein interactions [29].

The results of analysis of conserved residues in motifs **XI, XII, XIV, XV, and XVI** in proteins with presumed endonuclease activity (group 1) are shown in Table 2. Because the analyzed proteins were assigned to this group based solely on the presence of motif **XII** (Table 1), we used consensus sequences from Table 1 to check all group

1 proteins for the presence of other endonuclease motifs. Note that the consensus sequences were deduced based on the most conserved amino acids and did not guarantee identification of all the motifs. Thus, the motif **XII** consensus sequence (Table 1) was found in 81% sequences (1203 out of 1483) of the analyzed group. Because of this, we lowered the criteria for the consensus sequences by including in them only residues whose substitution would significantly decrease the MutL endonuclease activity [14]. The [D/E]XHXXE consensus was found in the corresponding region in 93.7% aligned sequences (1390 out of 1483); motif **XIV** (with Cys in the corresponding position) was found in 100% of these sequences (1390 out of 1390). Motif **XV** was found in 1373 out of 1390 sequences; functionally important Cys [14] of this motif was observed in 99.7% sequences (1386 out of 1390). The motif **XI** consensus was found in 89.4% sequences (1243 out of 1390).

Interestingly, none of 93 sequences without “weakened” motif **XII** consensus contained consensus sequences for motifs **XI, XIV, and XV**. At the same time, most of these sequences, as well as 1390 sequences with the “weakened” motif **XII** consensus, contained motif **XVI**. This corresponds well with the results of Kosinski et al. [14], who demonstrated strong correlation between the presence of all five motifs in the MutL sequence. They suggested that such correlation serves as evidence of a common functional activity that involves all these motifs. However, there exist MutL proteins that lack some of the endonuclease motifs [22].

Therefore, according to our data, group 1 MutL proteins containing motif **XII** in the *C*-terminal domain could be divided into two subgroups. The first includes 1390 proteins that contain all five endonuclease motifs; moreover, their motif **XII** contains all functionally characterized amino acid residues. These proteins possess endonuclease activity with a high degree of probability. The second subgroup includes 93 sequences that contain only motif **XII**, in which one of the functionally active residues is replaced with a residue with different properties. Proteins of this subgroup lack all other endonuclease motifs, and their properties are of particular interest.

The secondary structure of rsMutL was predicted based on the alignment of its amino acid sequence with sequences of MutL proteins with known spatial structures. To predict the secondary structure of the rsMutL *N*-terminal domain, we used the X-ray data obtained for ecMutL (PDB 1B63, 1NHI, 1BKN), PMS1 from *Saccharomyces cerevisiae* (PDB 3H4L), human PMS2 (PDB 1H7S), and human MLH1 (PDB 4P7A). To predict the secondary structure of the *C*-terminal domain, we used the structural data obtained for MutL from *B. subtilis* (PDB 3GAB, 3KDG, 3KDK), MutL from *A. aeolicus* (PDB 5B42), MutL from *N. gonorrhoeae* (PDB 3NCV), and MLH1/PMS1 heterodimer from *S. cerevisiae* (PDB 4E4W). In most cases, the elements of the secondary

structure in these sequences coincided. After comparing the sequences of rsMutL and its homologs using the Muscle software, we identified α -helical and β -strand regions in the MutL structure (indicated under rsMutL amino acid sequence in Fig. 1).

We found that rsMutL is most evolutionarily close to its homologs from *N. gonorrhoeae* (40% identity) and *P. aeruginosa* (44% identity). Both these microorganisms are proteobacteria that belong to different classes. MutL from *N. gonorrhoeae* (β -proteobacteria) is a homodimer with ATPase and endonuclease activities [30]. MutL from *P. aeruginosa* displays similar properties, even though

P. aeruginosa belongs to the γ -proteobacteria and does not possess MutH homologs [31]. We concluded that rsMutL of the *R. sphaeroides* DNA repair system should exhibit the DNA-hydrolyzing activity.

Purification of rsMutL. We amplified the *mutL* gene by PCR using *R. sphaeroides* genomic DNA as a template and cloned the resulting fragment into the pET15b vector containing the nucleotide sequence coding for the additional 36 a.a. including His₆ tag. The resulting His₆-linker-rsMutL plasmid was used for transformation of *E. coli* cells. The cells were grown in the presence of 100 μ g/ml ampicillin, and expression of the recombinant

Table 2. Conserved amino acid residues in endonuclease motifs XI, XII, XIV, XV, and XVI

Motif	Length, a.a.	Number of amino acid residue in rsMutL	Amino acid residue and its occurrence, %	Consensus
XI	2	432	A (45.1), G (45.6)	[G/A]Q
		433	Q (92.5)	
XII	12	451	D (94.9), E (4.4)	[D/E]XHXAXE[R/K][L/I/V]X[Y/F][E/D]
		452	variable; Q is the most common residue (73.4)	
		453	H (95.5)	
		454	variable; A is the most common residue (88.6)	
		455	A (98.4)	
		456	variable; H is the most common residue (82.0)	
		457	E (96.2)	
		458	R (97.1)	
		459	L (28.9), I (48.6), V (20.8)	
		460	variable	
		461	Y (79.6), F (11.6)	
462	E (94.8), D (2.7)			
XIV	3	562	variable; A is the most common residue (78.7)	C
		563	C (93.5)	
		564	variable; hydrophobic residues are more common (56.6)	
XV	6	594	C (93.5)	CXHX[R/K]P
		595	variable; N is the most common residue (51.5)	
		596	H (93.7)	
		597	variable	
		598	R (94.4), K (3.2)	
		599	P (95.6)	
XVI	3	613	F (97.6)	FX[R/K]
		614	variable	
		615	R (90.6), K (1.5)	

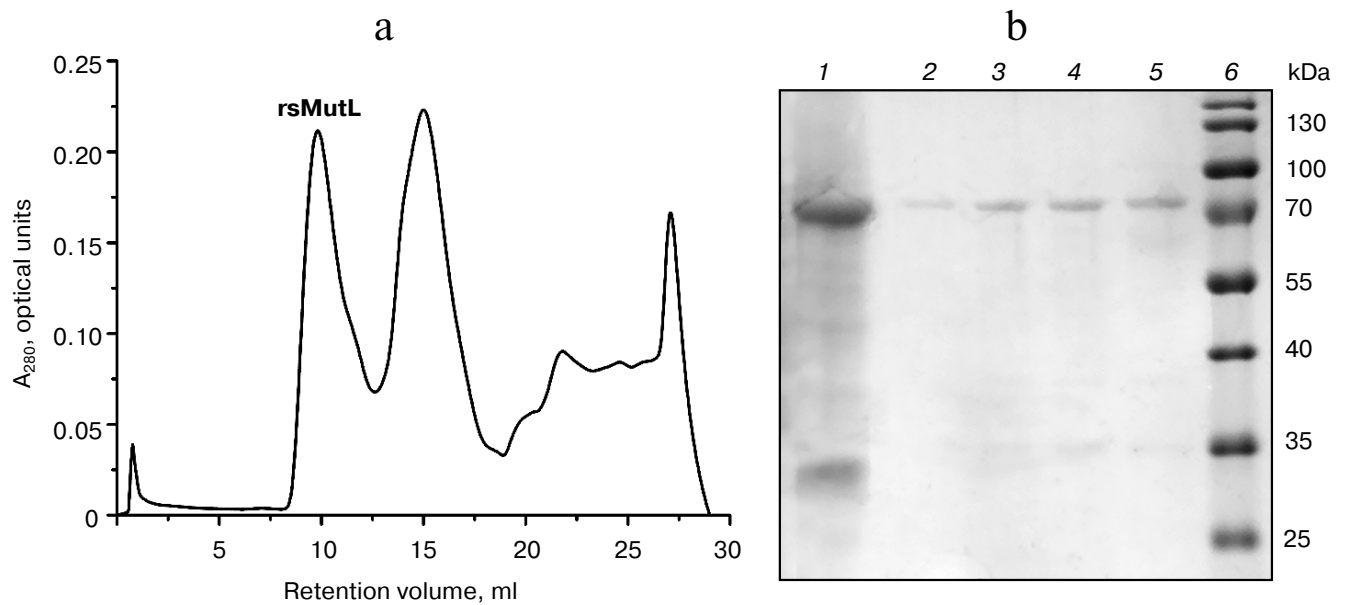


Fig. 2. rsMutL purification. a) Chromatographic separation of rsMutL from proteins with lower molecular masses. b) Analysis of fractions after size-exclusion chromatography: 1) MutL protein before chromatography; 2-5) fractions eluted with 9-10 ml; 6) 10-180 kDa molecular mass markers (Thermo Fisher Scientific).

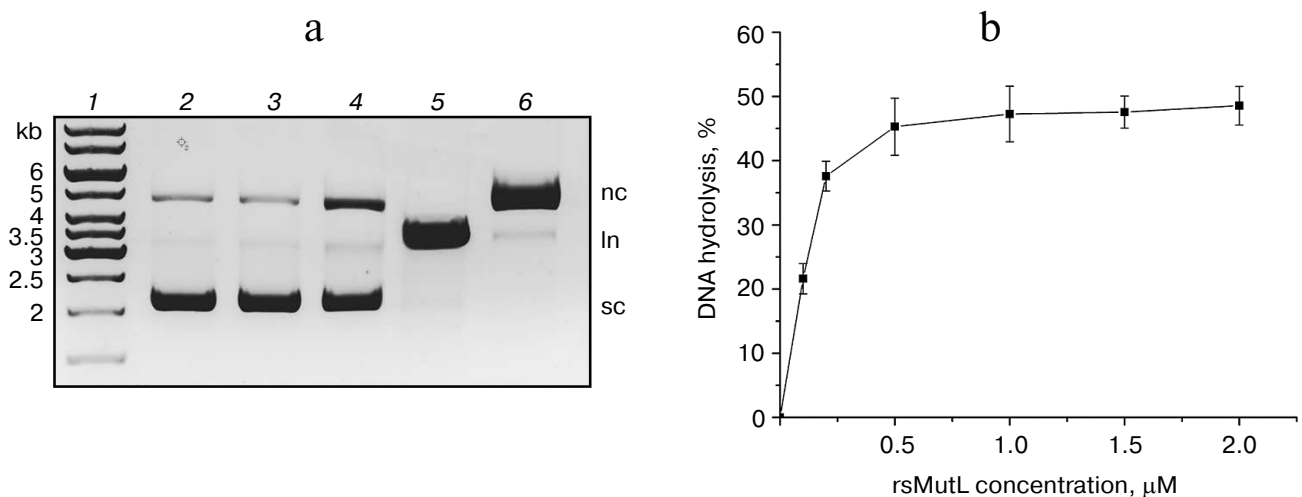


Fig. 3. Hydrolysis of the pUC-MMR plasmid by rsMutL at 37°C for 60 min. a) Electrophoresis in 1% agarose gel containing ethidium bromide: 1) DNA ladder (kb); 2) intact pUC-MMR; 3) pUC-MMR hydrolyzed by rsMutL in the absence of divalent metal cations; 4) pUC-MMR hydrolyzed by rsMutL in the presence of Mn^{2+} ; 5) pUC-MMR hydrolyzed by restriction endonuclease BamHI; 6) pUC-MMR hydrolyzed by nicking endonuclease Bpu10I; sc, supercoiled DNA; nc, nicked DNA; ln, linearized DNA. b) Dependence of the pUC-MMR (20 nM) hydrolysis on the rsMutL concentration (0-2 μM) in the presence of 5 mM $MnCl_2$.

protein was induced with IPTG. The aliquots of cells before and after induction were analyzed by Laemmli gel electrophoresis [32]. Because the recombinant protein contained an additional *N*-terminal sequence (MGSSHHHHHSSGLVPRGSHMGSRWSDSDSAKAGG) that included the His₆-tag separated from the rsMutL sequence by 26 a.a., recombinant rsMutL could be purified by affinity chromatography. It is commonly

believed that the presence of such additional sequence would not affect the properties of the modified protein, as it has been confirmed by protein crystallography data [33].

rsMutL was first purified by the method described for MutL from *N. gonorrhoeae* [16] that included cell lysis by sonication, centrifugation of the lysate, and chromatography of the resultant supernatant on Ni-NTA-agarose.

However, this procedure yielded only small amounts of homogenous rsMutL protein. The main problems were low efficiency of rsMutL binding to the sorbent, contamination of protein preparations with DNA, and low solubility of rsMutL. To improve rsMutL binding to Ni-NTA-agarose, we created two new plasmids containing optimized rsMutL gene. One of them coded for the rsMutL protein with the His₆-tag immediately at the *N*-terminus (His₆-rsMutL), the other with the His₆-tag immediately at the *C*-terminus (rsMutL-His₆). However, modifications of the *mutL* gene codon content for expression in *E. coli* and changes in the position of the His₆ tag failed to increase the protein yield (data not shown). Therefore, we modified the purification procedure. To promote correct protein folding, the concentration of IPTG was decreased to 0.1 mM, and protein expression was induced at decreased temperature (18°C) for 16–18 h. All solutions for rsMutL purification contained 0.05% Tween 20 to increase protein solubility and 1 M NaCl to prevent DNA binding to rsMutL.

rsMutL purified by the metal-chelating chromatography was separated from the products of its proteolysis and other proteins by size-exclusion chromatography on an ENrich SEC 650 column (10 × 300 mm; Bio-Rad) using an AKTA Explorer system (GE Healthcare Life Sciences, USA). Protein-containing fractions (elution volume, 9–12 ml; first peak) were collected, analyzed by Laemmli gel electrophoresis (Fig. 2), and concentrated. The yield of rsMutL was 0.2 mg (concentration, 8 μM).

DNA-hydrolyzing activity of rsMutL. To assay the rsMutL endonuclease activity, we used the pUC-MMR plasmid DNA. This plasmid was chosen because it can imitate the *in vivo* conditions, since the *R. sphaeroides* genome consists of two circular chromosomes and five plasmids [34]. It is also known that MutL proteins from *B. subtilis*, *N. gonorrhoeae*, *A. aeolicus*, *T. thermophilus*, and *P. aeruginosa* do not discriminate mismatches and hydrolyze canonical DNA in the absence of other MMR proteins [35]. All these MutL proteins display the maxi-

mum DNA-hydrolyzing activity in the presence of Mn²⁺ in the reaction mixtures [1].

The supercoiled pUC-MMR plasmid (10 or 20 nM) was incubated with rsMutL at 37°C in the presence or absence of MnCl₂. The concentration of rsMutL varied from 0 to 2 μM. The efficiency of DNA hydrolysis was estimated from the amount of formed nicked DNA (plasmid with a single-stranded break). The reaction mixture was analyzed by electrophoresis in 1% agarose gel containing ethidium bromide. pUC-MMR treated with the nicking endonuclease Bpu10I (nicked form) and restriction endonuclease BamHI (linearized form) was used as control. The nicked plasmid DNA has a lower mobility in the gel than the supercoiled DNA, while the mobility of the linearized form is between those of the supercoiled and nicked plasmid DNA (Fig. 3).

rsMutL did not hydrolyze pUC-MMR in the absence of divalent metal cations (Fig. 3). The presence of Mn²⁺ in the reaction mixture resulted in the formation of the nicked plasmid DNA. The efficiency of pUC-MMR hydrolysis increased with change in protein concentration from 0 to 0.5 μM and reached 50% at the rsMutL concentration of 1 μM. Further increase in the protein concentration did not influence on the yield of the hydrolyzed DNA. Also, the gel contained trace amounts of linearized DNA. Therefore, we demonstrated for the first time that rsMutL is an endonuclease capable of introducing single-single breaks in DNA.

Effect of divalent metal cations on DNA hydrolysis by rsMutL. The published data on the effect of divalent metals on the activity of MutL proteins from various organisms are contradictory [15–19] (Table 3).

We studied the efficiency of the pUC-MMR plasmid hydrolysis by rsMutL within 60 min in the presence of divalent cations that presumably modulate the endonuclease activity: Mg²⁺, Mn²⁺, Ca²⁺, Co²⁺, Ni²⁺, Cd²⁺, Zn²⁺ (Fig. 4). The efficiency of DNA cleavage decreased in the order Mn²⁺ > Co²⁺ > Mg²⁺ > Cd²⁺, while Ni²⁺, Ca²⁺, and Zn²⁺ produced no effect on the rsMutL endonuclease activity.

Table 3. Effect of metal ions and ATP on the endonuclease activity of MutL proteins from different microorganisms

Microorganism	Mn ²⁺	Mg ²⁺	Co ²⁺	Cd ²⁺	Ni ²⁺	Ca ²⁺	Zn ²⁺	ATP
<i>R. sphaeroides</i>	+	+	+	+	–	–	–	–
<i>N. gonorrhoeae</i>	+	+	ND	ND	ND	+	ND	–
<i>P. aeruginosa</i>	+	+	ND	ND	ND	ND	–	–
<i>B. subtilis</i>	+	–	ND	–	ND	ND	–	+*
<i>T. thermophilus</i>	+	ND	+	ND	+	ND	–	–
<i>A. aeolicus</i>	+	–	+	ND	+	–	–	+**

Note: +, stimulation of MutL endonuclease activity; –, no effect or inhibition (in the case of ATP) of MutL endonuclease activity; ND, not determined.

* Stimulation of endonuclease activity at 0.5 mM ATP; inhibition of endonuclease activity at 5 mM ATP [19].

** Effect of ATP on the MutL endonuclease activity depended on the concentrations of MutL, Mn²⁺, and ATP; stimulation was observed at the MutL concentration of 0.16–100 nM, 1 mM ATP, and 1 mM Mn²⁺ [45]; inhibition was observed at 100 nM MutL, 5 mM ATP, and 5 mM Mn²⁺ [18].

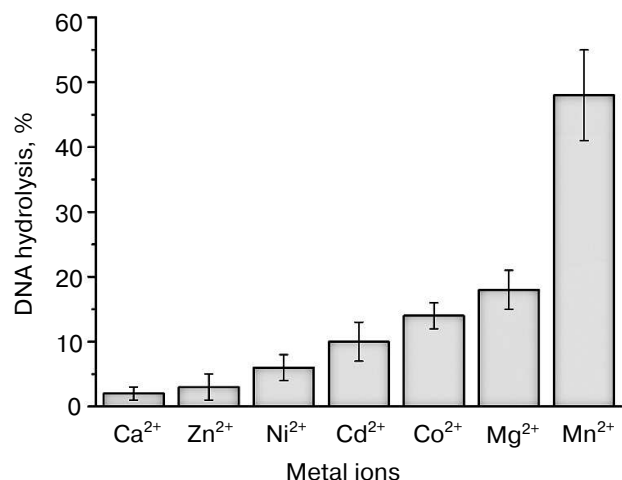


Fig. 4. Efficiency of pUC-MMR plasmid (20 nM) hydrolysis by rsMutL (1 μ M) in the presence of divalent metal cations (5 mM) at 37°C for 60 min.

Therefore, rsMutL most efficiently hydrolyzed plasmid DNA in the presence of MnCl₂, which correlates well with the data of other studies on DNA hydrolysis by MutL proteins from bacterial and human cells [1, 19]. It is known that many DNA polymerases use Mn²⁺ as a cofactor in DNA synthesis *in vitro*. Divalent manganese ion increases the enzyme activity, but it reduces fidelity and processivity of the polymerase [36, 37], which results in misincorporation of nucleotides in the DNA. Note that Co²⁺, like Mn²⁺ (but unlike other Me²⁺), increases the efficiency of DNA polymerases, although to a lesser extent, and promotes incorporation of incorrect nucleotides into DNA [38].

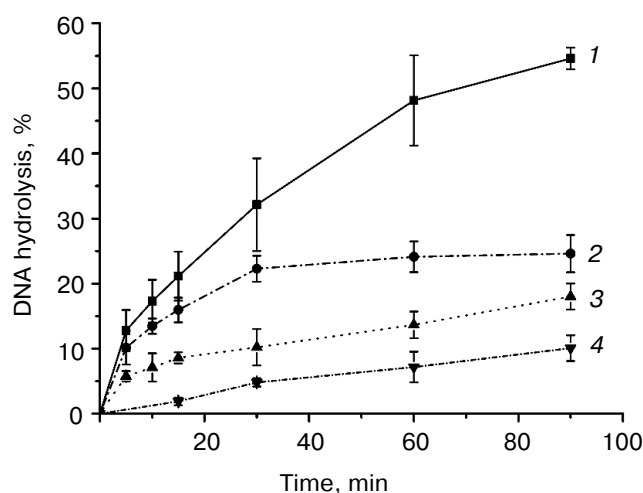


Fig. 5. Kinetics of pUC-MMR plasmid (20 nM) hydrolysis by rsMutL (1 μ M) in the presence of (1) 5 mM MnCl₂, (2) 5 mM MnCl₂ and 0.5 mM ATP, (3) 5 mM MgCl₂, and (4) 5 mM MgCl₂ and 0.5 mM ATP at 37°C.

Unlike Mn²⁺, Mg²⁺ is essential for the function of MutS, the first protein in the mismatch repair system, and formation of the MutS–MutL complex [39–42]. We investigated the kinetics of plasmid DNA cleavage by rsMutL in the presence of MgCl₂ and MnCl₂ (Fig. 5) and found that within the time interval of 0–90 min, Mn²⁺ was a more efficient cofactor in the DNA hydrolysis reaction than Mg²⁺. Since both MutL and MutS lose their activity when incubated at 37°C (data not shown), we limited the reaction time to 90 min. Note, DNA hydrolysis in the presence of either Mn²⁺ and Mg²⁺ proceeded with preferential formation of nicked DNA; however, trace amounts of linearized DNA were also formed, as follows from the presence of the corresponding low-intensity zones in the agarose gel (Fig. 3). Similar results were observed for MutL proteins from other bacteria [15–19].

As expected, Ca²⁺ did not activate DNA hydrolysis by rsMutL. For many endonucleases, Ca²⁺ promotes formation of the enzyme–substrate complex but blocks the hydrolytic activity of the enzyme [43, 44].

Divalent zinc ions play an important yet poorly understood role in MutL function. X-ray analysis showed [19] that MutL from *B. subtilis* coordinates Zn²⁺ not only in its active site, but also in a special Zn²⁺-binding domain. This coordination is provided by the side groups of Glu468, Cys604, and His606 residues, all of which are conserved in the MutL proteins with endonuclease activity. According to the alignment of the C-terminal domains from *B. subtilis* MutL and rsMutL (Fig. 1), these residues correspond to Glu457, Cys594, and His596 in rsMutL. In *B. subtilis* MutL, Zn²⁺ binding ensures convergence of amino acid residues in the enzyme active site. Interestingly, Zn²⁺ by itself does not stimulate hydrolysis of the supercoiled plasmid DNA by *B. subtilis* MutL;

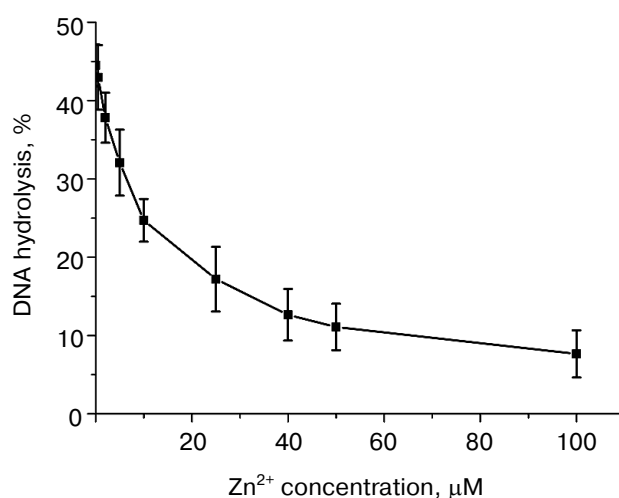


Fig. 6. Dependence of pUC-MMR plasmid (10 nM) hydrolysis by rsMutL (500 nM) on the concentration of Zn²⁺ (0–100 μ M) in the presence of 5 mM Mn²⁺ in the reaction mixture (37°C, 60 min).

however, the amount of the nicked and linearized DNA formed in the presence of equimolar concentrations of Zn^{2+} and Mn^{2+} was two times higher than in the presence of Mn^{2+} alone under the same conditions [19]. It was suggested that Zn^{2+} performs a structural function, similar to iron ions in the IdeR (iron-dependent repressors) proteins, whose primary and secondary structures are very similar to the structure of the C-terminal domains in MutL proteins with endonuclease activity [19]. On the other hand, Zn^{2+} activates MutL from *A. aeolicus* [18]. We found that Zn^{2+} did not promote DNA hydrolysis by rsMutL. On the contrary, addition of increasing Zn^{2+} concentrations (0-100 μ M) to the reaction mixture containing 5 mM Mn^{2+} inhibited DNA hydrolysis irrespectively of the Zn^{2+} concentration added (Fig. 6). Similar results were obtained for MutL for *P. aeruginosa* [15]. This suggests a high affinity of Zn^{2+} for the Me^{2+} -binding sites in rsMutL and the ability of Zn^{2+} to replace Mn^{2+} in them. Apparently, Zn^{2+} is not involved in DNA hydrolysis by rsMutL.

Therefore, divalent metal cations significantly affect the ability of rsMutL to introduce single-stranded breaks in DNA and its specificity.

Effect of ATP on MutL endonuclease activity. As mentioned above, the presence of characteristic conserved motifs in the rsMutL sequence indicates that rsMutL belongs to the family of GHKL ATPases. ATP binding and hydrolysis might cause significant conformational changes in the protein molecule. Indeed, ATP was found to affect the endonuclease activity of MutL, although the results of these studies are quite contradictory (Table 3). ATP strongly activates DNA hydrolysis by human MutL α . Low ATP concentrations stimulate the activity of MutL from *B. subtilis*, while high ATP concentrations inhibit the enzyme [19]. Similarly, ATP can either stimulate [45] or inhibit [18] MutL from *A. aeolicus* depending on the ATP concentration, enzyme concentration, and the presence of Mg^{2+} . ATP inhibits DNA hydrolysis by MutL proteins from *N. gonorrhoeae*, *T. thermophiles*, and *P. aeruginosa* [15-17].

In this work, we analyzed the effect of ATP on the rsMutL activity. In most studies on DNA hydrolysis by MutL, the concentration of ATP is 0.5 mM [18, 30]. We found that addition of 0.5 mM ATP to the reaction mixture containing Mn^{2+} or Mg^{2+} decreased the extent of plasmid DNA hydrolysis approximately two times when the reaction was carried out for 5 to 90 min (Fig. 5), which correlates well with the data obtained for MutL enzymes from *N. gonorrhoeae*, *A. aeolicus*, *T. thermophiles*, and *P. aeruginosa*. Inhibition of MutL endonuclease activity by ATP might be important for cell functioning. The intracellular concentration of ATP is approximately 3-5 mM [17], which is high enough to considerably decrease the ability of MutL to hydrolyze DNA, thereby preventing introduction of undesirable breaks in the canonical DNA.

In conclusion, we purified for the first time the MutL protein, a component of the mismatch repair system from *R. sphaeroides*, and demonstrated its endonuclease activity. Comparison of rsMutL with its analogs from *A. aeolicus*, *B. subtilis*, *N. gonorrhoeae*, *T. thermophilus*, and *P. aeruginosa* showed that all these proteins hydrolyze DNA in the presence of Mn^{2+} (Table 3). However, only rsMutL and MutL from *N. gonorrhoeae* introduce single-stranded breaks in DNA in the presence of Mg^{2+} and Co^{2+} . Divalent zinc ions do not stimulate DNA cleavage by any of the MutL proteins studied, which indicates the necessity for ion coordination for the active protein structure formation. ATP significantly inhibits the DNA-hydrolyzing activity of rsMutL and all other enzymes presented in Table 3 (except MutL from *B. subtilis*). Analysis of MutL amino acid sequences revealed functionally important conserved motifs (Tables 1 and 2) in these proteins. Considering all these data, MutL from *R. sphaeroides* mostly resembles MutL proteins from *N. gonorrhoeae* and *P. aeruginosa* in its primary structure and biochemical properties.

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REFERENCES

1. Guarne, A. (2012) The functions of MutL in mismatch repair: the power of multitasking, *Prog. Mol. Biol. Transl. Sci.*, **110**, 41-70.
2. Iyer, R. R., Pluciennik, A., Burdett, V., and Modrich, P. L. (2006) DNA mismatch repair: functions and mechanisms, *Chem. Rev.*, **106**, 302-323.
3. Jiricny, J. (2013) Postreplicative mismatch repair, *Cold Spring Harb. Perspect. Biol.*, **5**, a012633.
4. Lynch, H. T., Lynch, P. M., Lanspa, S. J., Snyder, C. L., Lynch, J. F., and Boland, C. R. (2009) Review of the Lynch syndrome: history, molecular genetics, screening, differential diagnosis, and medicolegal ramifications, *Clin. Genet.*, **76**, 1-18.
5. Michailidi, C., Papavassiliou, A. G., and Troungos, C. (2012) DNA repair mechanisms in colorectal carcinogenesis, *Curr. Mol. Med.*, **12**, 237-246.
6. Lahue, R. S., and Modrich, P. (1988) Methyl-directed DNA mismatch repair in *Escherichia coli*, *Mutat. Res.*, **198**, 37-43.
7. Längle-Rouault, F., Maenhaut-Michel, G., and Radman, M. (1987) GATC sequences, DNA nicks and the MTH function in *Escherichia coli* mismatch repair, *EMBO J.*, **6**, 1121-1127.

8. Modrich, P. (1989) Methyl-directed DNA mismatch correction, *J. Biol. Chem.*, **264**, 6597-6600.
9. Perevozchikova, S. A., Romanova, E. A., Oretskaya, T. S., Friedhoff, P., and Kubareva, E. A. (2013) Modern concepts of the structural and functional organization of the system for the DNA noncanonical nucleotide pair repair, *Acta Naturae*, **5**, 27-44.
10. Hall, M. C., Shcherbakova, P. V., and Kunkel, T. A. (2002) Differential ATP binding and intrinsic ATP hydrolysis by amino-terminal domains of the yeast Mlh1 and Pms1 proteins, *J. Biol. Chem.*, **277**, 3673-3679.
11. Sancar, A., and Hearst, J. E. (1993) Molecular matchmakers, *Science*, **259**, 1415-1420.
12. Kadyrov, F. A., Dzantiev, L., Constantin, N., and Modrich, P. (2006) Endonucleolytic function of MutL α in human mismatch repair, *Cell*, **126**, 297-308.
13. Kadyrov, F. A., Holmes, S. F., Arana, M. E., Lukianova, O. A., O'Donnell, M., Kunkel, T. A., and Modrich, P. (2007) *Saccharomyces cerevisiae* MutL α is a mismatch repair endonuclease, *J. Biol. Chem.*, **282**, 37181-37190.
14. Kosinski, J., Plotz, G., Guarne, A., Bujnicki, J. M., and Friedhoff, P. (2008) The PMS2 subunit of human MutL α contains a metal ion binding domain of the iron-dependent repressor protein family, *J. Mol. Biol.*, **382**, 610-627.
15. Correa, E. M. E., Martina, M. A., De Tullio, L., Argaran, C. E., and Barra, J. L. (2011) Some amino acids of the *Pseudomonas aeruginosa* MutL D(Q/M)HA(X)(2)E(X)(4)E conserved motif are essential for the *in vivo* function of the protein but not for the *in vitro* endonuclease activity, *DNA Repair*, **10**, 1106-1113.
16. Duppatla, V., Bodda, C., Urbanke, C., Friedhoff, P., and Rao, D. N. (2009) The carboxy-terminal domain is sufficient for endonuclease activity of *Neisseria gonorrhoeae* MutL, *Biochem. J.*, **423**, 265-277.
17. Fukui, K., Nishida, M., Nakagawa, N., Masui, R., and Kuramitsu, S. (2008) Bound nucleotide controls the endonuclease activity of mismatch repair enzyme MutL, *J. Biol. Chem.*, **283**, 12136-12145.
18. Iino, H., Kim, K., Shimada, A., Masui, R., Kuramitsu, S., and Fukui, K. (2011) Characterization of C- and N-terminal domains of *Aquifex aeolicus* MutL endonuclease: N-terminal domain stimulates the endonuclease activity of C-terminal domain in a zinc-dependent manner, *Biosci. Rep.*, **31**, 309-322.
19. Pillon, M. C., Lorenowicz, J. J., Uckelmann, M., Klocko, A. D., Mitchell, R. R., Chung, Y. S., Modrich, P., Walker, G. C., Simmons, L. A., Friedhoff, P., and Guarne, A. (2010) Structure of the endonuclease domain of MutL: unlicensed to cut, *Mol. Cell*, **39**, 145-151.
20. Bijlsma, J. J., and Groisman, E. A. (2003) Making informed decisions: regulatory interactions between two-component systems, *Trends Microbiol.*, **11**, 359-366.
21. Capra, E. J., and Laub, M. T. (2012) Evolution of two-component signal transduction systems, *Annu. Rev. Microbiol.*, **66**, 325-347.
22. Banasik, M., and Sachadyn, P. (2014) Conserved motifs of MutL proteins, *Mutat. Res.*, **769**, 69-79.
23. Ban, C., and Yang, W. (1998) Crystal structure and ATPase activity of MutL: implications for DNA repair and mutagenesis, *Cell*, **95**, 541-552.
24. Dutta, R., and Inouye, M. (2000) GHKL, an emergent ATPase/kinase superfamily, *Trends Biochem. Sci.*, **25**, 24-28.
25. Plotz, G., Raedle, J., Brieger, A., Trojan, J., and Zeuzem, S. (2003) N-terminus of hMLH1 confers interaction of hMutL α and hMutL β with hMutS α , *Nucleic Acids Res.*, **31**, 3217-3226.
26. Pillon, M. C., Miller, J. H., and Guarne, A. (2011) The endonuclease domain of MutL interacts with the beta sliding clamp, *DNA Repair (Amst.)*, **10**, 87-93.
27. Davis, R. W., Botstein, D., and Roth, J. R. (1980) *Advanced Bacterial Genetics: a Manual for Genetic Engineering*, Cold Spring Harbor, Cold Spring Harbor Laboratory, N.Y.
28. Winkler, I., Marx, A. D., Lariviere, D., Heinze, R. J., Cristovao, M., Reumer, A., Curth, U., Sixma, T. K., and Friedhoff, P. (2011) Chemical trapping of the dynamic MutS-MutL complex formed in DNA mismatch repair in *Escherichia coli*, *J. Biol. Chem.*, **286**, 17326-17337.
29. Echave, J., Spielman, S. J., and Wilke, C. O. (2016) Causes of evolutionary rate variation among protein sites, *Nat. Rev. Genet.*, **17**, 109-121.
30. Namadurai, S., Jain, D., Kulkarni, D. S., Tabib, C. R., Friedhoff, P., Rao, D. N., and Nair, D. T. (2010) The C-terminal domain of the MutL homolog from *Neisseria gonorrhoeae* forms an inverted homodimer, *PloS One*, **5**, 13726.
31. Jacquelin, D. K., Filiberti, A., Argaraca, C. E., and Barra, J. L. (2005) *Pseudomonas aeruginosa* MutL protein functions in *Escherichia coli*, *Biochem. J.*, **388**, 879-887.
32. Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature*, **227**, 680-685.
33. Carson, M., Johnson, D. H., McDonald, H., Brouillette, C., and Delucas, L. J. (2007) His-tag impact on structure, *Acta Crystallogr. D. Biol. Crystallogr.*, **63**, 295-301.
34. Choudhary, M., Mackenzie, C., Nereng, K. S., Sodergren, E., Weinstock, G. M., and Kaplan, S. (1994) Multiple chromosomes in bacteria: structure and function of chromosome II of *Rhodobacter sphaeroides* 2.4.1, *J. Bacteriol.*, **176**, 7694-7702.
35. Ban, C., Junop, M., and Yang, W. (1999) Transformation of MutL by ATP binding and hydrolysis: a switch in DNA mismatch repair, *Cell*, **97**, 85-97.
36. Lakhin, A. V., Efremova, A. S., Makarova, I. V., Grishina, E. E., Shram, S. I., Tarantul, V. Z., and Gening, L. V. (2013) Effect of Mn(II) on the error-prone DNA polymerase iota activity in extracts from human normal and tumor cells, *Mol. Genet. Mikrobiol. Virusol.*, **1**, 14-20.
37. Frank, E. G., and Woodgate, R. (2007) Increased catalytic activity and altered fidelity of human DNA polymerase iota in the presence of manganese, *J. Biol. Chem.*, **282**, 24689-24696.
38. Zakharcheva, K. A., Gening, L. V., Kazachenko, K. Yu., and Tarantul, V. Z. (2017) Cells resistant to toxic concentrations of manganese have increased ability to repair DNA, *Biochemistry (Moscow)*, **82**, 101-110.
39. Grilley, M., Welsh, K. M., Su, S. S., and Modrich, P. (1989) Isolation and characterization of the *Escherichia coli* mutL gene product, *J. Biol. Chem.*, **264**, 1000-1004.

40. Prolla, T. A., Pang, Q., Alani, E., Kolodner, R. D., and Liskay, R. M. (1994) MLH1, PMS1, and MSH2 interactions during the initiation of DNA mismatch repair in yeast, *Science*, **265**, 1091-1093.
41. Drotschmann, K., Aronshtam, A., Fritz, H. J., and Marinus, M. G. (1998) The *Escherichia coli* MutL protein stimulates binding of Vsr and MutS to heteroduplex DNA, *Nucleic Acids Res.*, **26**, 948-953.
42. Acharya, S., Foster, P. L., Brooks, P., and Fishel, R. (2003) The coordinated functions of the *E. coli* MutS and MutL proteins in mismatch repair, *Mol. Cell*, **12**, 233-246.
43. Mordasini, T., Curioni, A., and Andreoni, W. (2003) Why do divalent metal ions either promote or inhibit enzymatic reactions? The case of BamHI restriction endonuclease from combined quantum-classical simulations, *J. Biol. Chem.*, **278**, 4381-4384.
44. Pingoud, A., Fuxreiter, M., Pingoud, V., and Wende, W. (2005) Type II restriction endonucleases: structure and mechanism, *Cell Mol. Life Sci.*, **62**, 685-707.
45. Mauris, J., and Evans, T. C. (2009) Adenosine triphosphate stimulates *Aquifex aeolicus* MutL endonuclease activity, *PLoS One*, **4**, 71-75.