Observation of The Interaction Between MutS and MutL Mismatch Repair Proteins by Fusion Protein Systems*

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Abstract MutL and MutS or their homologues are two crucial proteins of DNA mismatch repair (MMR) system. A new method was described for observation of the interaction between MutS and MutL which is based on the fusion gene/fusion protein technique. Three fusion proteins, MutL-GFP fusion (Trx-His_6-GFP-$(Ser\text{-}Gly)_6$-MutL), MutL-Strep tagⅡ fusion (Trx-His_6-$(Ser\text{-}Gly)_6$-Strep tagII-$(Ser\text{-}Gly)_6$-MutL) and MutS fusion (Trx-His_6-$(Ser\text{-}Gly)_6$-MutS), were constructed and expressed in *E.coli* AD494 (DE3). Interaction assay between MutS and MutL was performed in a 96-well microtiter plate. MutS fusion protein was immobilized on the wells and provided a surface for the interaction between MutS and MutL. Results showed that only after binding of MutS to the mismatched DNA, there was an interaction between MutS and MutL. The binding events could be indicated by GFP signal or the signal generated from alkaline phosphatase and its substrate. In addition, the method based on fusion molecular system also serve as a model for studies on the interactions among other proteins or biomolecules.

Key words fusion protein, GFP, strep tagⅡ, MutS, MutL, interaction

DNA mismatch repair (MMR) system exists widely from prokaryotes to eukaryotes and the human. They serve a vital function to correct DNA biosynthetic errors that arise during chromosomal replication and to discourage recombination between substantially diverged DNA sequences [1]. Thus, an active mismatch repair system ensures the precision of chromosomal replication, maintains genomic stability, and decreases mutation rate and the incidence of various cancers. For example, in the human, the overwhelming majority of hereditary nonpolyposis colorectal cancers (HNPCC), a number of familial non-HNPCC, and sporadic cancers have been attributed to mutations in the genes encoding MutS and MutL homologues[2, 3].

The MMR system is composed of a family of proteins, such as MutS, MutL and MutH or their homologues [4]. MutS, MutL and MutH have been characterized individually in previous studies [5-8] and interactions between these MMR proteins become an extensive interest to the investigators [9-11]. Based on some findings, a few hypotheses have been proposed to describe the mechanism of MMR system [12]. According to a model of MMR proposed by Schofield[13], after binding of MutS to the mismatched region of DNA, MutL immediately acts as a central coordinator to stop MutS slipping away from the mismatch site, so as to enhance such binding and stimulate the endonuclease activity of MutH. To further understand details of the mechanism, more effective experiment methods are to be explored. The aim of this paper is to build a reliable method for direct observation of interaction between MutS and MutL proteins. The method is essentially based on the gene fusion/protein fusion technique. Figure 1 demonstrates the scheme of the design. MutS fusion protein is pre-immobilized on the solid surface and binds the mismatched DNA. MutL was genetically labeled with green fluorescence protein (GFP) or Strep tagⅡ that subsequently binds to the strepavidin-alkaline phosphatase fusion. From the signal of the GFP or enzymatic reaction of alkaline phosphatase, it is easy to "see" the contribution of MutL to the binding efficiency of MutS to the mismatched DNA.

*This work was supported by grants from The National Natural Science Foundation of China (30500097), Ministry of Science & Technology and The Chinese Academy of Science (KSCX1-06-01).

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Received: June 13, 2005 Accepted: August 28, 2005

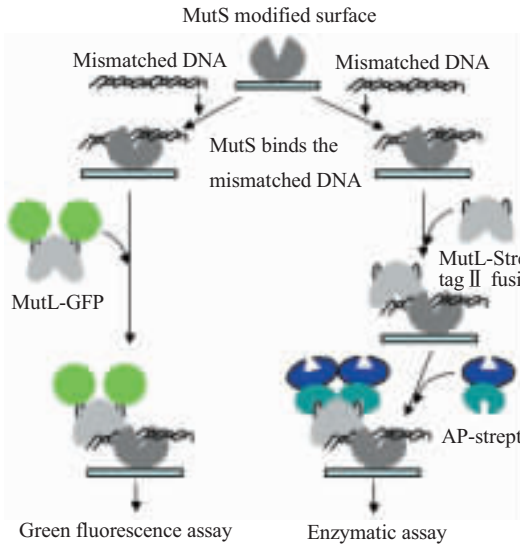

Fig. 1 The schematic representation of the interaction of MutS and MutL fusion

There are two experiment schemes: left, GFP scheme; right, enzymatic scheme.

1 Materials and methods

1.1 Materials

E.coli AD494 (DE3) and plasmid pET32a (+) were from Novagen Company. PCR primers used for cloning *mutL* gene and $(Ser\text{-}Gly)_6$ encoding sequence are synthesized by Sangon Company (Shanghai, China). The vector pGEM-Ti was from Promega Company. Ampicillin, Kanamycin sulphate, bovine serum albumin (BSA), isopropylthio-β-D-galactoside (IPTG) and 5-bromo-4-chloro-3-indolyl-β-D-galacto-pyronoside (X-gal) were purchased from Sigma Company. Restriction enzymes, DNA polymerase, and T4 DNA ligase were from Takara Company and Promega Company. PCR purification mini kit and gel extraction mini kit were from Huaxun Company. *E.coli* DH5α used for all bacterial transformations and plasmid propagations and *E.coli* K-12 was stored in our laboratory. ALON metal affinity resins were purchased from Novagen Company. All other reagents were of analytical-reagent grade.

1.2 Molecular cloning of the MutL encoding sequence

Molecular cloning of the MutL encoding sequence has been described in our previous publication[7].

1.3 Construction of expression systems of MutL fuion proteins

The plasmid pGEM-T-*mutL* was digested with *Eco*R Ⅰ and *Hin*d Ⅲ, and MutL encoding fragment was recovered. GFP encoding fragment was obtained by digesting plasmid pGEM-T-*gfp* with *Bgl* Ⅱ and *Eco*R Ⅴ. $(Ser\text{-}Gly)_6$ encoding sequence was produced by annealing L1 (5′ATCAGCGGCTCAGGATCTGG-ATCAGGATCTGGCG 3′) and L2 (5′AATTCGCC-AGATCCTGATCCAGATCCTAGCCGCTGAT 3′) at 70℃ for 5 min to form dsDNA fragment with *Eco*R Ⅴ and *Eco*R Ⅰ cutting sites at its N and C-termini, respectively. GFP encoding fragment, $(Ser\text{-}Gly)_6$ encoding sequence and MutL encoding fragment were then inserted to replace the corresponding sequence between *Bgl* Ⅱ and *Hin*d Ⅲ in the vector pET32a (+), and yielded the fusion expression vector pET32a-*gfp*-$(Ser\text{-}Gly)_6$-*mutL*.

The expression vector of MutL-strep tag Ⅱ fusion was constructed similarly. S1 (5′GATCTGAGCGGC-TCTGGATCAGGATCTGGCAGCGCTTGGAGCCA CCCGCAGTTCGAAAAAGGCGCCGAT 3′) and S2 (5′ ATCGGCGCCTTTTTCGAACTGCGGGTGGCT-CCAAGCGCTGCCAGATCCTGATCCAGAGCCGC TCA 3′) was first annealed at 70℃ for 5 min to form Strep tag Ⅱ encoding sequence with *Bgl* Ⅱ and *Eco*R Ⅴ at its N and C-termini, respectively. The $(Ser\sim Gly)_6$ and Strep tag Ⅱ encoding fragment were then inserted to replace the corresponding sequence between *Bgl* Ⅱ and *Eco*R Ⅴ in the vector pET32a-*gfp*-$(Ser\text{-}Gly)_6$-*mutL*, and yielded the expression vector pET32a-$(Ser\text{-}Gly)_6$-*Strep tag* Ⅱ-$(Ser\text{-}Gly)_6$-*mutL*.

1.4 Expression and purification of MutL fusion proteins

The fusion protein expression vectors, pET32a-*gfp*-$(Ser\text{-}Gly)_6$-*mutL* and pET32a-$(Ser\text{-}Gly)_6$-*Strep tag* Ⅱ-$(Ser\text{-}Gly)_6$-*mutL*, were transformed into the host *E.coli* AD494 (DE3), separately, and yielded the recombinant strains AD494 (DE3)/ pET32a-*gfp*-$(Ser\text{-}Gly)_6$-*mutL* and AD494 (DE3)/pET32a-$(Ser\text{-}Gly)_6$-*Strep tag* Ⅱ-$(Ser\text{-}Gly)_6$-*mutL*. A single colony of the strains from a fresh LB plate containing ampicillin and kanamycin sulphate was inoculated into 10 ml of LB liquid medium containing the same antibiotics, respectively. The culture was incubated with rotary shaking (300 r/min) at 37℃ for 6~8 h to mid-exponential phase. Cells were collected by centrifugation at 5 000 *g* for 20 min and the pellet was resuspended in 1 ml of fresh LB medium. 1 ml of this suspension was inoculated to 1 000 ml of LB medium containing the same antibiotics. The culture was allowed to grow with rotary shaking (300 r/min) at 37℃ for 4 h before 100 mmol/L IPTG were added and continue to incubate at 28℃ or 30℃ for 5 h. Cultures

were then chilled on ice for 10 min, and cells were collected by centrifugation. After washing twice with 100 ml of ice-cold water, the cell pellets were resuspended in 10 ml of 1 ×extraction /wash buffer (50 mmol/L sodium phosphate, 300 mmol/L NaCl, pH 7.0) per 100 ml of cell culture and lysed by sonication. Lysates were centrifugated at 12 000 *g* for 30 min. The supernatant was harvested and loaded onto His-Bind resin column pre-equilibrated with 10 column volumes of 1 × extraction/wash buffer. The column was washed with 10 column volumes of 1 × extraction/wash buffer.The desired protein, MutL-GFP or MutL-strep tag Ⅱ, was eluted by washing the resin with 1× elution buffer (50 mmol/L sodium phosphate, 300 mmol/L NaCl, 150 mmol/L imidazole, pH 7.0). The elution buffer containing the purified MutL-GFP or MutL-strep tag Ⅱ was dialyzed overnight against 1 000 ml of dialyzing buffer (50 mmol/L Tris-HCl, pH 7.2, 100 mmol/L KCl, 1 mmol/L DTT and 1 mmol/L EDTA).

1.5 SDS-PAGE Analysis

Concentration and purity of MutL fusions were determined using Bio-Rad Assay Kit with BSA as the standard and SDS-PAGE, respectively. The collected cells and purified proteins were all treated with 2 × sample buffer. After boiling for 5 min, the sample was loaded onto a slab of 10% polyacrylamide gel. The gel was run in 1 × TAE buffer at 10 V/cm at room temperature, and then was stained with Coomassie Brilliant Blue G-250.

1.6 Characterization of MutL fusions

Characterization of MutL fusions was performed by band-shift assays using MutS fusion protein Trx-His_6-$(Ser\text{-}Gly)_6$-MutS constructed in our previous study[14] and short duplex DNA fragment containing a mismatched base pair and complementary DNA fragment according to the bioactivity of MutL stimulating the binding of the protein MutS to DNA[7]. The synthesized oligonucleotides 5′ GTGTCCAGA-TCCGTCXACCTGAGAACTATT 3′ were mixed with the corresponding oligonucleotides. The mixture was heated and slowly cooled to room temperature to form a duplex DNA containing GT mismatch at X site and complementary DNA. Binding reaction mixture contained 1 μl of duplex DNA, 39 μl of Millipore water, 5 μl of 10×assay buffer (200 mmol/L Tris-HCl, pH 7.6, 50 mmol/L $MgCl_2$, 1 mmol/L DTT, and 0.1 mmol/L EDTA) and MutS fusion together with MutL-strep tag Ⅱ fusion or MutL-GFP fusion at different concentrations. The reaction mixture was kept on ice for 30 min before 20 μl of 50% sucrose was added. The resulting mixture was loaded onto 6% nondenaturing polyacrylamide gel in low-ionic strength buffer (7 mmol/L Tris-HCl, 3.3 mmol/L NaAc, 1 mmol/L EDTA, pH 7.9). The electrophoresis was performed at 10 V/cm at 4℃ until the bromophenol blue dye band in the control lane migrated approximately 6 cm. Since it is difficult to estimate the amount of duplex DNA bound to the MutS fusion together with MutL-strep tag Ⅱ fusion or MutL-GFP fusion directly, the relative amount of unbound duplex DNA, as an indication of the bioactivity of MutL-strep tag Ⅱ fusion or MutL-GFP fusion, was quantified by using Bio Imaging System.

The Strep tag Ⅱ bioactivity assay of the fusion protein has been described in our previous publication[15].

1.7 Interaction of the fusion protein MutL-strep tag Ⅱ fusion or MutL-GFP fusion with MutS fusion

There were two experiment schemes. In scheme Ⅰ, the interaction of the MutL-strep tag Ⅱ fusion with MutS was performed on a 96-well microtiter plate. A 200 μl aliquot of 0.4 g/L MutS fusion solution was applied to each well and incubated at 4℃ overnight. After removal of the solution, the wells were blocked with 3% (*w*/*v*) BSA-0.5% (*v*/*v*) Tween 20, 2 g/L salmon sperm DNA solved in the buffer B (20 mmol/L Tris-HCl, pH 7.6, 5.0 mmol/L $MgCl_2$, 0.1 mmol/L DTT and 0.01 mmol/L EDTA) for 30 min, followed by washing three times with buffer B-Tween 20 (0.1% Tween 20). Then 30 μl aliquot of 3 mg/L mismatched duplex DNA or complementary DNA was added to the binding reaction mixture and kept on the ice for 30 min. Unbound DNA was removed by washing three times with buffer B. Finally, 0.3 g/L MutL-strep tag Ⅱ fusion solved in the buffer C (20 mmol/L Tris-HCl, pH 7.6, 5.0 mmol/L $MgCl_2$, 0.02 mmol/L ATP, 0.1 mmol/L DTT and 0.01 mmol/L EDTA) was added to the wells, unbound MutL-strep tag Ⅱ fusion was removed by washing with buffer C. 100 μl of 100 mg/L streptavidin-alkaline phosphatase conjugate was then added and incubated for 0.5 h. Unbound conjugate was removed by washing twice with PBS-Tween 20 and twice with PBS buffer, separately. The solution BCIP/NBT, the substrate of the alkaline phosphatase conjugate, was applied to each well. The enzymatic reaction was allowed to perform at room temperature for 20 min. The interaction between MutL and MutS was then evaluated directly according to color change.

In scheme Ⅱ, the interaction of the fusion protein

MutL-GFP with MutS was observed directly through emission of GFP of the MutL-GFP fusion. The fluorescence intensity was measured with Synergy HT Multi-Detection Microplate Reader.

2 Results

2.1 Expression of MutL fusion proteins

The fusion proteins were over-expressed by inducing with IPTG under the control of a T7-phage promoter. The maximum yields of MutL-strep tag Ⅱ fusion was obtained when the culture was induced with 1.0 mmol/L IPTG and then continued to grow at 30℃ for 5 h, while the maximum yield of MutL-GFP fusion was obtained by growing at 28℃ after induction. Figure 2 shows the total proteins of the cell and purified proteins. The protein bands corresponding to MutL-GFP and MutL-strep tag Ⅱ were visualized at 109 ku and 86 ku, respectively. The desired proteins reached about 30% of the total cellular protein and the purity was over 90% after purification.

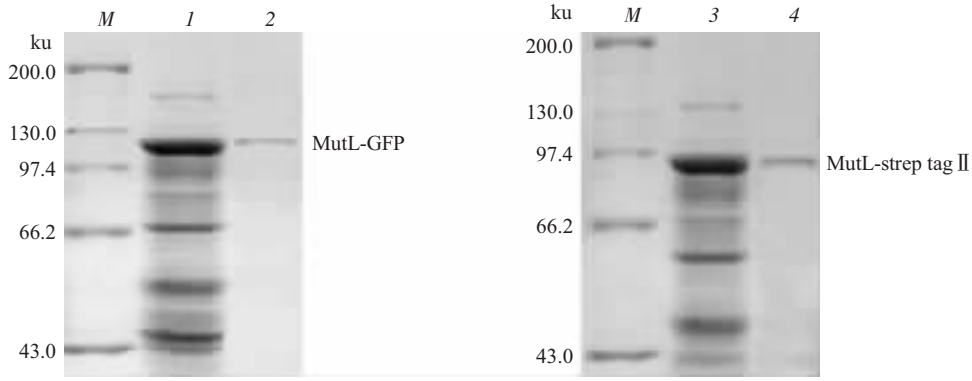

Fig. 2 SDS-PAGE assay of MutL-GFP fusion and MutL-strep tag Ⅱ fusion

M: High molecular mass marker; *1*: The fusion protein MutL-GFP before purifying; *2*: Purified fusion protein MutL-GFP; *3*: The fusion protein MutL-strep tag Ⅱ before purifying; *4*: Purified fusion protein MutL-strep tag Ⅱ.

2.2 Characterization of MutL fusion proteins

MutL-bioactivities of the MutL fusion proteins were determined by checking the amount decrease of unbound duplex DNA during non-denaturing polyacrylamide gel. The mixture of MutS, MutL-GFP and duplex DNA with a single-base mismatch was incubated on ice for 30 min. Another mixture without MutL-GFP was prepared as a control. As shown in lane *1* and *3* of Figure 3, neither MutL-GFP nor MutS could apparently bind paired DNA because all DNA run into their position on the gel. In lane *4* brightness of the DNA band decreased and many of the DNA molecules could not migrated into the right position, which implies the DNA molecules were bound by MutS. In lane *2* the DNA band disappeared, which is the evidence of markedly enhanced binding of MutS to the mismatched DNA by co-existence of MutL-GFP. But addition of MutL-GFP or MutL-strep tag Ⅱ to the binding reaction mixture did not produce any detectable super-shifted or intermediate band, as shown in Figure 3. This suggests the transient or unstable association of MutL-GFP/MutS-DNA complex and MutL-strep tag Ⅱ/MutS-DNA complex, or the complex hardly entering the gel matrix because of their high molecular mass.

Fig. 3 The bioactivity of MutL-GFP fusion stimulating MutS binding to DNA

1: MutS together with MutL-GFP fusion plus paired DNA; *2*: MutS together with MutL-GFP fusion plus mismatched DNA; *3*: MutS plus paired DNA; *4*: MutS plus mismatched DNA.

2.3 Interaction between MutL and MutS

As shown in Figure 1, The MutS modified surfaces were employed for the study of the interaction between MutL-strep tag Ⅱ and MutS directly. The detailed procedure is described in the experimental section. Figure 4 shows the results of the enzymatic scheme. The contents of each well of the microtiters are given in the figure legend, each had two duplicates. After addition of streptavidin-alkaline phosphatase fusion and the enzyme substrate sequentially, at the given time (20 min), only the wells (No.*6*) that

contained MutS fusion protein, mismatched DNA, MutL-strep tag Ⅱ and ATP developed brown color. This is the strong indication of the interaction of MutL and MutS in existence of ATP. Without ATP and mismatched DNA there was no detectable color change. Most of the control experiments developed color gradually after the addition of streptavidin-alkaline phosphatase conjugate and its substrate. Therefore, it is critical to limit the reaction time strictly. Long incubation time (> 20 min) might cause pseudo positive signal due to the partial nonspecific absorption of MutL-strep tag Ⅱ or the nonspecific absorption of streptavidin-alkaline phosphatase conjugate.

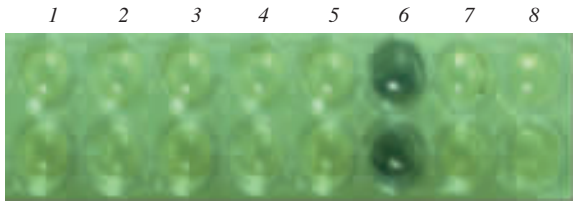

Fig. 4 The interaction between the fusion protein MutL-strep tag Ⅱ and MutS (Enzymatic reaction scheme)

1: MutL-strep tag Ⅱ plus mismatched DNA; *2*: MutS together with BSA plus mismatched DNA; *3*: MutS plus MutL-strep tag Ⅱ; *4*: MutS together with BSA and ATP plus mismatched DNA; *5*: MutS together with MutL-strep tag Ⅱ plus mismatched DNA; *6*: MutS together with MutL-strep tag Ⅱ and ATP plus mismatched DNA; *7*: Only DNA; *8*: Only MutL-strep tag Ⅱ.

Experiment protocol of the GFP scheme is similar to that of the enzymatic scheme, except that there was no addition of enzyme fusion and enzyme substrate, the signal was directly from GFP that was fused with MutL. As shown in Figure 5, the pixel intensity from the negative controls, were between 2 569 and 4 978, while the signal intensity of the mixture of MutS fusion, mismatched DNA, MutL-GFP fusion and ATP

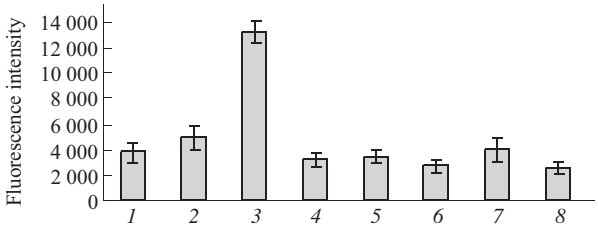

Fig. 5 The interaction between the fusion protein MutL-GFP and MutS (Green fluorescence assay scheme)

1: MutL-GFP plus mismatched DNA; *2*: MutS together with MutL-GFP plus mismatched DNA; *3*: MutS together with MutL-GFP and ATP plus mismatched DNA; *4*: MutS together with BSA and ATP plus mismatched DNA; *5*: Only MutL-GFP; *6*: MutS together with BSA plus mismatched DNA; *7*: MutS plus MutL-GFP; *8*: Only DNA.

reached 13 296, which is 5.2~2.7 folds increase over the negative control, giving a distinguished signal. This is again the direct evidence of the interaction between MutL and MutS in the presence of mismatched DNA and ATP.

3 Discussion

Compare to MutS protein, the function of MutL is less characterized. It seems to be an interface between MutS and MutH, playing a role to stimulate the sequence-specific endonuclease activity of MutH [9], and it is clear that MutL catalyzes a weak ATPase reaction required for mismatch repair [16]. Some investigators considered that MutL is an ATP-independent binding protein with affinity for both ssDNA and dsDNA [17, 18], but such binds are rather weak[19], whereas, in this study, we did not find obvious association of MutL alone with DNA. In fact, most current models for DNA mismatch repair do not invoke direct MutL interaction with the DNA substrate. Sancar and Hearst suggested that MutL is an example of the molecular matchmaker class of proteins and causes an ATP-dependent conformational change in one or more DNA binding protein partners to promote protein-DNA interaction[20]. The interaction between MutS and MutL requires both mismatched duplex DNA and ATP, but the analysis of the complex is not easy. Fragmentary data have been obtained from electron microscopy, immunoprecipitation, band shifts and plasmon resonance experiments. Grilley, *et al* [5] reported that *E.coli* MutL interacted with MutS-hDNA in an ATP-dependent manner to enlarge the footprint obtained with MutS alone using DNAse Ⅰ protection experiments. Interaction of *E.coli* MutS and MutL with heteroduplex DNA has been visualized by electron microscopy [12]. Mark suggested that a MutS-MutL complex formed in the vicinity of a mismatch and is involved in activating MutH protein[13].

In the present study, two experiment schemes were adopted, GFP fluorescence and alkaline phosphatase catalytic reaction, all gave the same evidence that there is an interaction between MutL and MutS in the existence of ATP and mismatched DNA. The method is straightforward and versatile. With the MutS-modified wells, one could easily apply manifold experiment conditions for study of interaction of MutS and MutL and the influence of the various factors, and the results could be read out directly. For example, it was found that MutL can stimulate the binding of MutS to the mismatched DNA at the range of 5~

30 μmol/L of ATP concentration (data not shown). Besides, since MutL is a dimmer, one molecule of MutL-GFP fusion bears two molecules of GFP, the fusion can thus generate stronger signal.

In the constructed molecular fusion systems, thioredoxin was used as a fusion partner in order to circumvent the formation of inclusion bodies caused by the over-expression of heterologous gene and His. tag was fused to facilitate the purification of the desired proteins[21]. A flexible linker peptide composed of serine and glycine repeats was designed to minimize the steric hindrance between the functional components of the fusion protein. This linker peptide had been used in our previous studies[22,23] and proved to be successful again here because all components of the fusion proteins MutL-strep tag Ⅱ and MutL-GFP fusion, i.e. thioredoxin, His.tag, GFP, strep tag Ⅱ and MutL, maintained their native functions.

References

1 Modrich P, Lahue R. Mismatch repair in replication fidelity, genetic recombination, and cancer biology. Annu Rev Biochem, 1996, **65**: 101~133

2 Kolodner R D. Mismatch repair: mechanisms and relationship to cancer susceptibility. Trends Biochem Sci, 1995, **20** (10): 397~401

3 Matic I, Taddei F, Radman M. Genetic barriers among bacteria. Trends Microbiol, 1996, **4** (2): 69~72

4 Lu A L, Clark S, Modrich P. Methyl-directed repair of DNA base-pair mismatches *in vitro*. Proc Natl Acad Sci USA, 1983, **80** (15): 4639~4643

5 Grilley M, Welsh K M, Su S S, *et al*. Isolation and characterization of the Escherichia coli mutL gene product. J Biol Chem, 1989, **264** (2): 1000~1004

6 Bi L J, Zhou Y F, Deng J Y, *et al*. High Expression and Identification of DNA Mismatch Repair Gene mutS in *E.coli*. Chin J Biotech, 2002, **18** (5): 536~540

7 Bi L J, Zhou Y F, Zhang X E, *et al*. High expression of gene encoding for MutL fusion protein and research on its chaperon function. Chin J Biochem Mol Biol, 2004, **20** (2): 149~155

8 Joseph N, Sawarkar R, Rao D N. DNA mismatch correction in Haemophilus influenzae: characterization of MutL, MutH and their interaction. DNA Repair (Amst), 2004, **3** (12): 1561~1577

9 Hall M C, Matson S W. The *Escherichia coli* MutL protein physically interacts with MutH and stimulates the MutH-associated endonuclease activity. J Biol Chem, 1999, **274** (3): 1306~1312

10 Mechanic L E, Frankel B A, Matson S W. *Escherichia coli* MutL loads DNA helicase Ⅱ onto DNA. J Biol Chem, 2000, **275** (49): 38337~38346

11 Plotz G, Raedle J, Brieger A, *et al*. N-terminus of hMLH1 confers interaction of hMutLalpha and hMutLbeta with hMutSalpha. Nucleic Acids Res, 2003, **31** (12): 3217~3226

12 Allen D J, Makhov A, Grilley M, *et al*. MutS mediates heteroduplex loop formation by a translocation mechanism. EMBO J, 1997, **16** (14): 4467~4476

13 Schofield M J, Nayak S, Scott T H, *et al*. Interaction of *Escherichia coli* MutS and MutL at a DNA mismatch. J Biol Chem, 2001, **276** (30): 28291~28299

14 Bi L J, Zhou Y F, Zhang X E, *et al*. Construction and characterization of different MutS fusion proteins as recognition elements of DNA Chip for detection of DNA mutations. Biosensors and Bioelectronics, 2005, **21** (1): 135~144

15 Bi L J, Zhou Y F, Zhang X E, *et al*. A MutS based protein chip for detection of DNA mutations. Anal Chem, 2003, **75**: 4113~4119

16 Spampinato C, Modrich P. The MutL ATPase is required for mismatch repair. J Biol Chem, 2000, **275** (13): 9863~9869

17 Ban C, Junop M, Yang W. Transformation of MutL by ATP binding and hydrolysis: a switch in DNA mismatch repair. Cell, 1999, **97**(1): 85~97

18 Bende S M, Grafstrom R H. The DNA binding properties of the MutL protein isolated from *Escherichia coli*. Nucleic Acids Res, 1991, **19** (7): 1549~1555

19 Drotschmann K, Aronshtam A, Fritz H J, *et al*. The *Escherichia coli* MutL protein stimulates binding of Vsr and MutS to heteroduplex DNA. Nucleic Acids Res, 1998, **26** (4): 948~953

20 Sancar A, Hearst J E. Molecular matchmakers. Science, 1993, **259** (5100): 1415~1420

21 Richard R D, Robert A E, Joseph B, *et al*. Versatile *E.coli* thioredoxin specific monoclonal antibodies afford convenient analysis and purification of prokaryote expressed soluble fusion protein. J Immunol Methods, 1995, **185** (2): 237~244

22 Shao W H, Zhang X E, Liu H, *et al*. Anchor-chain molecular system for orientation control in enzyme immobilization. Bioconjug Chem, 2000, **11** (6): 822~826

23 Zhou Y F, Zhang X E, Liu H, *et al*. Construction of a fusion enzyme system by gene splicing as a new molecular recognition element for a sequence biosensor. Bioconjug Chem, 2001, **12** (6): 924~931

DNA错配修复蛋白 MutS 和 MutL 的相互作用研究 *

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摘要 MutL 和 MutS 是 DNA 错配修复系统中起关键作用的修复蛋白. 利用基因融合技术高效表达了 MutL 和 MutS 融合蛋白，并利用它们发展了一种研究二者相互作用的简便方法. 融合蛋白 MutL-GFP (Trx-His_6-GFP-$(Ser\text{-}Gly)_6$-MutL)，MutL-Strep tagⅡ (Trx-His_6-$(Ser\text{-}Gly)_6$-Strep tagⅡ-$(Ser\text{-}Gly)_6$-MutL) 和 MutS (Trx-His_6-$(Ser\text{-}Gly)_6$-MutS) 被构建并在大肠杆菌中高效表达. 收集菌体细胞、超声波破碎后离心取上清进行 SDS- 聚丙烯酰胺凝胶电泳 (SDS-PAGE) 分析，结果表明有与预期分子质量相应的诱导表达条带出现，其表达量约占全细胞蛋白的 30%且以可溶形式存在. 利用固定化金属离子配体亲和层析柱分别纯化融合蛋白，其纯度达到 90%. 通过将 MutS 蛋白固定的方法研究两种 MutL 融合蛋白分别与 MutS 之间的相互作用. 结果表明：只有 MutS 蛋白与含有错配碱基 DNA 分子结合后才与 MutL 蛋白发生相互作用. 通过检测 MutL 融合蛋白标记的绿色荧光信号或酶学显色信号来鉴定相互作用的发生. 建立的融合分子系统方法也为研究其他的蛋白质或生物大分子之间的相互作用提供了一个技术平台.

关键词 融合蛋白，绿色荧光蛋白，Strep tagⅡ，MutS，MutL，相互作用

学科分类号 Q7

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*国家自然科学基金资助项目(30500097), 国家“九五”攻关项目和中国科学院“九五”重大项目(KSCX1-06-01).

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收稿日期：2005-06-13, 接受日期：2005-08-28