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SYNERGISM OF DAM, MUTH, and MUTS in METHYLATION-DIRECTED MISMATCH REPAIR IN *ESCHERICHIA COLI*

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Abstract

DNA mismatch repair (MMR) is a critical mutation surveillance system for recognizing and repairing erroneous insertion, deletion, and disincorporation of base. Major components of mismatch repair system consist of MutH, MutL, and MutS. Dam methylates adenine to distinguish newly synthesized daughter strands from the parent strands. Employing a tyrosine-auxotrophic *E. coli* FX-11 strain, the mutation frequency can be determined by the number of tyrosine revertants and the cell viability of FX-11 with deficiencies in *dam* and mismatch repair proteins. This study showed that *mutS* defect produced a higher mutation frequency than *mutH* did. Interestingly, double defects in *dam* and *mutS* synergistically produced a dramatically higher spontaneous mutation frequency than the summation of mutation frequencies of FX-11 strains with individual deficiency of *dam* or *mutS*, suggesting that Dam may work with MutHL to partially accomplish the task of recognizing the mismatch sites to retain partial mismatch repair capacity.

Keywords

DNA methylation-directed mismatch repair; MutS; MutH; Dam

Introduction

Regardless of the powerful proofreading capacity of DNA polymerases, mistakes naturally occur in the newly synthesized daughter strand during DNA replication (10^{-7} per bp per replication). DNA mismatch repair (MMR) is an essential mutation surveillance system for

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#equally contribute to this work.

Conflict of interest

No conflict of interest is declared.

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identifying and repairing incorrect insertion, deletion, and disincorporation of bases, which further elevates fidelity by 50–1000 folds, keeping the low mutation rate (1 per 10^9 – 10^{10} base pairs per cell division) and maintaining the genome integrity [1] [2].

In addition to replication errors, genetic recombination can also result in mismatches when the heteroduplex intermediate contains nucleotide sequence differences between the recombining DNA molecules. The mismatches can also be processed by mismatch repair system [3].

Contribution of mismatch repair system to replication fidelity depends on the ability to discriminate strands and direct the repair on the newly synthesized DNA strand. DNA methylase (Dam) adds a methyl group to the adenine of the sequence 5'-GATC-3' in newly synthesized DNA [4]. Immediately after DNA synthesis, the daughter strand remains unmethylated for a short time [5]. Mismatch repair occurs on the non-methylated strand of hemi-methylated DNA, which is newly synthesized DNA strand. The hemi-methylated GATC site subsequently provides a strand break, which directs the mismatch repair event [6].

MMR is a highly conserved molecular mechanism that plays an indispensable role in both prokaryotes and eukaryotes. MMR consists of three important protein components, MutS, MutH, and MutL, which play critical roles in identifying and repairing the mismatch. MutS recognizes the mismatched base pairs and initiates the mismatch repair [7]. Then MutL is recruited to the MutS - DNA heteroduplex complex in an ATP-dependent fashion [8]. The assembly of the MutS-MutL-heteroduplex ternary complex activates MutH, which incises the unmethylated strand of a hemi-methylated GATC site 5' to the G [9]. UvrD helicase (DNA helicase II) is then recruited to unwind the heteroduplex with favored direction from the strand break to the mismatched pair. Depending on which side MutH incises the strand in relative to the mismatch (5' or 3'), different groups of exonucleases will be recruited to excise the sequence from MutH incision to the mismatched site [10]. DNA Polymerase III fills the gap, and the nick is linked by the DNA ligase. Dam then rapidly methylates the daughter strand.

E. coli strains defective in *mutH*, *mutL*, or *mutS* are deficient in methyl-directed mismatch repair and display elevated spontaneous mutation frequencies [11]. In addition, since the heteroduplex DNA without the methylation by Dam on either strand is also processed but with little or no strand bias, *E. coli* cells deficient in Dam methylase are also mutators [12]. It has been shown that the combination of multiple defects in mismatch repair system (*mutS*, *mutH*, *mutL*) leads to additive effect on producing spontaneous mutations [11], however, it is poorly understood how the combination of *dam* deficiency and any of defective mismatch repair proteins affects the process of mismatch repair and the production of mutation. In this study, we employed an *E. coli* tyrosine revertant system to investigate how the *dam* deficiency change the spontaneous mutation frequencies of *E. coli mutS* or *mutH* mutant. This work sheds light on the possible interaction and cooperation between Dam methylase and mismatch repair proteins to accomplish the task of mistake repair.

2. Materials and Methods

2.1 Bacterial Strains

The bacterial strains employed in this research are listed in Table 1. *E. coli* strain FX-11 was derived from *E. coli* B/r WU 3610, which is auxotrophic for tyrosine (a UAA nonsense defect) and leucine (a UAG nonsense defect), and contain a UAG-suppressing, glutamine-inserting tRNA mutation (*glnV^a = supE*). FX-11 was excision repair defective (*uvrA115*) and has been previously described [13]. The Uvr- sensitivity was confirmed by UV-sensitivity.

Strain FX-11 *dam*, FX-11 *mutS* and FX-11 *mutH* was constructed by P1 transduction from *E. coli* GM 2151 (*dam-13::Tn9*), ES1481 (*mutS215::Tn10*) and JW2799-2 (*mutH756::kan*), respectively.

2.2 Mutagenesis Assay

The mutagenesis assay was carried out by selecting Tyr⁺ revertants that are capable of growing in the absence of added tyrosine in medium. These revertants can be produced by two fundamentally different ways. First, the TAA in the *tyrA14* gene defect may be changed to specify an appropriate amino acid such as glutamine (UAA to CAA), tyrosine (UAA to UAU, UAC), lysine (UAA to AAA), etc. This site in the protein is particularly flexible to amino acid substitution, and all but one (TAA to TGA) possible single base change occurring at these specific A:T base pairs allow the production of functional polypeptide. These changes are referred to as ‘backmutations’. Alternatively, mutation may occur in a gene encoding one of several different transfer RNA molecules so that the anti-codon of a tRNA is changed to 3′-AUU-5′, allowing recognition of the UAA nonsense codon during translation. As a consequence, an amino acid is inserted at the unaltered UAA defect, producing a functional polypeptide. These mutations are termed “suppressor” mutations.

The procedure for the isolation of revertants has been described [14]. Briefly, overnight cultures were grown in A-0 minimal media containing limiting glucose (0.04%) and tyrosine (20 µg/ml). Bacteria were then diluted 1:20 in A-0 minimal media with full glucose (0.4%) and tyrosine. Bacterial cells were grown to an OD₄₅₀ = 0.5 (a density 2–3×10⁸ cells/ml), centrifuged and re-suspended in A-0 buffer. The number of spontaneous mutations, represented by reversions to tyrosine prototrophy, was determined by plating cells onto A-0 semi-enriched minimal agar plates containing 0.02% nutrient broth and leucine (20 µg/ml each) but lacking tyrosine. These plates were incubated at 37 °C for 48 h and the number of revertants was counted. Viability was determined by plating appropriately diluted suspensions (10⁻⁶) of bacterial cells onto A-0 semi-enriched minimal agar plates and colonies were counted after 24 hours incubation at 37 °C. The frequency of spontaneous mutations of each strain was determined by the formula: Mutation Frequency = Y/N, where the variables N and Y represent the number of viable cells (per ml) and the number of mutants (per ml), respectively. The experiment for each strain was repeated for three times and the mutation frequency was represented as mean ± SD.

3. Results and Discussion

The tyrosine auxotrophic *E. coli* FX-11 and its mutant strains with deficiencies in *dam*, *mutS*, *mutH*, *dam* and *mutS*, or *dam* and *mutH* were plated onto A-0 minimal media containing limiting tyrosine to determine the number of revertants. The frequency of spontaneous mutation was determined by the ratio between the number of revertants and the viability. The spontaneous mutation frequencies of FX-11 strains are shown in Figure 1. Since defective Dam cannot methylate adenine in either DNA strand and thus mismatch repair system is not able to discriminate for the daughter strand on which the error occurs, as expected, *E. coli* cells deficient in *dam* displayed an elevated spontaneous mutation frequency (more than 2-fold). In addition to *dam* deficiency, *E. coli* strains that over-produce Dam are also hypermutable because excessive Dam activity reduces the temporal window during which the daughter strand has not been methylated and mismatch repair occurs [15]. Both *mutS* and *mutH* mutations are strong mutators and remarkably increased the spontaneous mutation frequencies by 21.8- and 15.6-fold, respectively. The spontaneous mutation frequency of *mutS* mutant was significantly higher than that of *mutH* mutant. The stronger mutating character of *mutS* mutant emphasizes the importance of recognition of mismatches by MutS as the initiation of mismatch repair process. The addition of *dam* deficiency to either *mutS* or *mutH* mutant led to significantly higher spontaneous mutation frequency than that of *mutS* or *mutH* mutant alone. Remarkably, FX-11 *dam* & *mutS* showed a dramatically higher spontaneous mutation frequency than the summation of mutation frequencies of FX-11 strains with individual deficiency of *dam* or *mutS*, suggesting that deficiencies in *dam* and *mutS* synergistically cause the production of mutations. Since a functional Dam in FX-11 *mutS* can reduce the mutation productions of the double mutant (FX-11 *dam* & *mutS*) by a magnitude that is much more than the mutation produced by *dam* deficiency alone, we have a contemplative thought that Dam methylase may work with MutHL to partially address the problem of recognizing the mismatch site in an unknown mechanism to keep the mismatch system work at incomplete capacity. Alternatively, Dam methylase may participate in an MMR-independent repair mechanism. The third plausible hypothesis is, instead of recognizing the newly synthesized strand by the lack of methylation and initiating the mismatch repair on this strand in the conventional pathway, MutS could achieve initial mismatch recognition in a Dam-independent way, possibly by associating with DNA replication machinery. In this model, since both the conventional pathway and the proposed mechanism require Muts, but Dam is only required in one of them (the conventional pathway), existence of this proposed mechanism could explain the relatively low mutation frequency observed in *dam* deficiency and the higher mutation rate observed in *mutS* deficiency. The interaction, interplay and cross-talk between Dam methylase and mismatch repair proteins, and the cooperation between the strand discrimination machinery and the mismatch repair system need further investigation. Worth to mention, this study employed an *E. coli* tyrosine revertant system, in which the “backmutation” and “suppressor mutation” occurs on the UAA nonsense codon of *tyrA14* gene and the 3'-AUU-5' anticodon of tRNA genes (allowing recognition of the UAA nonsense codon during translation), respectively. Therefore, the mutation spectrum is limited by the genetic context of relevant genes. The mutation frequencies of *dam* and *mutS/mutL/mutH* mutants should be determined in more genetic contexts to examine the generality.

4. Conclusion

The *E. coli* mutants with deficiency in *dam*, *mutH* and *mutS* are hypermutable. The spontaneous mutation frequency of *mutS* mutant was significantly higher than that of *mutH* mutant. The combination of *dam* deficiency and defective mismatch repair proteins (*dam* & *mutS* or *dam* & *mutH*) produced remarkably higher spontaneous mutation frequency than *dam* or *mutS/mutH* did alone. The deficiencies in *dam* and *mutS* synergistically increased the spontaneous mutation frequencies, implying that Dam methylase may work with MutHL to somewhat recognize the mismatch site in an unknown mechanism.

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Figure 1. Spontaneous mutation frequencies of *E. coli* FX-11 and mutant strains

The spontaneous mutation frequencies of FX-11 and mutant strains were determined by calculating the ratios between the number of revertants and the viability of all the six different strains. The mutation frequencies are represented by mean $\pm$ SD. * $P < 0.05$; ** $P < 0.01$.

Table 1*Escherichia coli* strains

<i>Escherichia coli</i> strain	Relevant Genotype	Donor strain	Source
FX-11	<i>tyrA14, supE, uvrA115</i>		D. Fix
FX-11 <i>dam</i>	as FX-11, <i>dam-13::Tn9</i> (Chloramphenicol ^R)	GM 2151	CGSC#6578
FX-11 <i>mutS</i>	as FX-11, <i>mutS215::Tn10</i> (tetracycline ^R)	ES1481	CGSC#7049
FX-11 <i>mutH</i>	as FX-11, <i>mutH756::kan</i> (kanamycin ^R)	JW2799-2	CGSC#10186
FX-11 <i>dam</i> & <i>mutS</i>	as FX-11, <i>dam-13::Tn9</i> (Chloramphenicol ^R) & <i>mutS215::Tn10</i> (tetracycline ^R)	GM 2151 and ES1481	CGSC#6578 & CGSC#7049
FX-11 <i>dam</i> & <i>mutH</i>	as FX-11, <i>dam-13::Tn9</i> (Chloramphenicol ^R) & <i>mutH756::kan</i> (kanamycin ^R)	GM 2151 and JW2799-2	CGSC#6578 & CGSC#10186