

GEN 02592

Specificity of the Dam-directed mismatch repair system of *Escherichia coli* K-12*

(Recombinant DNA; methylation; proofreading; *mut* genes; hotspots; plasmid pBR322; phage P22)

M. Carraway, C. Rewinski, T.-H. Wu and M.G. Marinus

Department of Pharmacology, University of Massachusetts Medical School, Worcester, MA 01655 (U.S.A.)

Received 25 May 1988

Accepted 9 June 1988

Received by publisher 22 June 1988

Spontaneous base substitution mutations in *Escherichia coli* probably arise mainly as the consequence of errors introduced into DNA during replication. At least two mechanisms are known which remove base pair mismatches from DNA: proofreading and post-replicative Dam-directed mismatch repair. The *mutD* gene, which specifies the ϵ -subunit of DNA polymerase III holoenzyme, has been implicated in proofreading since mutant alleles confer a strong mutator phenotype.

The Dam-directed mismatch repair system removes errors in the newly synthesized daughter DNA strand. The discrimination between daughter and parental DNA strands resides in the differential state of methylation of 5'-GATC-3' (Dam recognition) sequences. The *dam* locus of *E. coli* specifies a DNA adenine methyltransferase which modifies the adenine residue in 5'-GATC-3' sequences. In *dam* mutant strains, which have un-

methylated DNA, strand discrimination for repair is lost, and half the time the repair mechanism should fix the replication error into the DNA sequence and lead to an increased mutation frequency.

Strains of *E. coli* which are deficient in Dam-directed mismatch repair should also show a mutator phenotype. The products of the *mutH* and *mutL* genes, among others, are required for efficient repair of mismatched heteroduplexes. In such *mut* strains the mutation spectrum should be identical to that found in the *dam-3* strain if the model for Dam-directed repair is correct.

The target sequence we have used to monitor spontaneous mutations is the *mnt* repressor gene of bacteriophage P22 which is present in cells cloned into a pBR322 vector derivative. We found that the mutation spectra in *dam*, *mutL* and *mutH* bacteria were all the same. In these cells transition mutations predominated indicating that A/C and/or G/T mismatches were corrected preferentially in wt bacteria. We have also found that AT to GC mutations clustered at three sites ('hotspots') and that these sites are within 6 bp of the only two GATCs in the target gene. The mechanism by which the hotspots are generated and their relationship to the Dam-recognition sites is under investigation.

In contrast to the strains defective in Dam-directed mismatch repair, *mutD* cells show a substantial frequency of transversion mutations of the type AT to TA and AT to CG, as well as transition mutations. Most of the transversion mutations occur within one

Correspondence to: Dr. M.G. Marinus, Department of Pharmacology, University of Massachusetts Medical School, 55 Lake Ave., Worcester, MA 01655 (U.S.A.) Tel. (508)856-3330.

* Presented at the New England Biolabs Workshop on Biological DNA modification, Gloucester, MA (U.S.A.) 20-23 May 1988.

Abbreviations: bp, base pair(s); Dam, DNA adenine methyltransferase; *dam*, gene coding for Dam; IS, insertion sequence; *mnt*, bacteriophage P22 gene involved in maintenance of lysogeny; *mut*, gene whose mutant allele results in a mutator phenotype; wt, wild type.

of the Dam-recognition sequences. We are currently trying to determine if it is the GATC per se or the methylation of this sequence which promotes the hotspot.

Repair by proofreading and Dam-directed mismatch repair must be efficient since IS1 induced mutations are found to predominate in wt cells.

REFERENCES

- Carraway, M., Youderian, P. and Marinus, M.G.: Spontaneous mutations occur near Dam recognition sites in a *dam*⁻ *Escherichia coli* host. *Genetics* 116 (1987) 343-347.
- Rewinski, C. and Marinus, M.G.: Mutation spectrum in an *Escherichia coli* DNA mismatch repair deficient (*mutH*) strain. *Nucleic Acids Res.* 15 (1987) 8205-8215.

Edited by S. Hattman.