Biochemical Pharmacology, Vol. 37, No. 9, pp. 1811–1812, 1988.
Printed in Great Britain.

0006-2952/88 $3.00 + 0.00
Pergamon Press plc

SINGLE AMINO ACID CHANGES WHICH ALTER THE SEQUENCE SPECIFICITY OF THE T4 (DAM) DNA-ADENINE METHYLTRANSFERASE. Zoe Miner, Samuel Schlagman and Stanley Hattman, University of Rochester, Department of Biology, Rochester, NY 14627.

Many enteric bacteria contain a DNA adenine methyltransferase (Dam) that methylates the A residue in the sequence, GATC. The related T-even phages, T2 and T4 (but not T6), also encode Dam methylases (1); the normal substrate for these enzymes is 5-hydroxymethylcytosine (hmC)-containing DNA, since these viruses contain this base in place of C, and the hmC is modified further by glucosylation. Nonglucosylating mutants (gt-) have been isolated that are different from their gt+ parents in that they are unable to grow on certain strains, such as P1 lysogenic hosts. Derivatives of T2 gt- and T4 gt- phage capable of growth on P1 lysogens have been isolated; these are designated dam^h because they exhibit hypermethylation of their DNA. Thus, Dam^h, but not Dam+, methylation protects against P1 restriction of the asymmetric sequence, AGACC.

To begin to understand the nature of Dam+ and Dam^h methylation, we cloned a functional T4 dam+ gene (2) and its nucleotide sequence was established (3). To date our attempts to clone the T4 dam^h gene have been unsuccessful; this may be due to the fact that E. coli mrr+ cells were used for transformation, and mrr+ strains restrict DNA with m6A in certain sequences other than GATC (4). However, second-site mutants of dam^h, designated dam^h dam-x, can be isolated, which are unable to methylate phage DNA (5). Taking advantage of these, we have cloned and sequenced two independent T4 dam^h dam-x mutants. They share a common amino acid (Pro to Ser) substitution at residue 126; and they each have a frameshift mutation (+1 or -1) at different sites farther downstream. Furthermore, a portion of the dam gene from a T2 dam^h dam-x mutant was cloned and sequenced; this mutant exhibits the same Pro to Ser alteration at amino acid 126. Therefore, we propose that this Pro to Ser alteration is a consequence of the the dam+ ---> dam^h mutation; it is interesting that this residue is within one of three high homology regions common to three prokaryotic Dam methylases (6). Finally, a spontaneous plasmid mutant in T4 dam+ was found that methylates C-DNA, but not hmC-DNA. This mutant has a single base substitution altering amino acid 127 (Phe to Val). Thus, two different single amino acid substitutions at adjacent sites alter the methylation capability of the Dam enzyme.

We have recently succeeded in creating a nonsense UAG at codon 126 in a cloned T4 dam+ gene. Introducing another plasmid with a cloned supD gene, which inserts Ser at amber codons, results in temperature dependent suppression. Thus, DNA methylation occurs at 30°C, but not 42°C. Having such an amber-dam^h mutant will allow us to take advantage of a series of vectors with different cloned sup genes. By separately introducing each sup vector, whose suppression at UAG is known, the effect on DNA methylation of known amino acids at residue 126 can be evaluated. Initial experiments have shown that an extract from supD cells (grown at 42°C) containing the amber-dam^h mutant suppressor is able to add additional methyl groups to purified T4 dam+ DNA *in vitro*.

T2 Dam appears to have a lower sequence specificity than T4 Dam; i.e. T2 Dam methylates some subset of GAPy. We have cloned and sequenced the T2 dam gene and compared it to the known sequence of the T4 dam gene. The two are almost identical, for there are only 3 amino acid differences. These are at codon 20 [T2(Leu) vs T4(Ser)], codon 26 [T2(Asp) vs T4(Asn)] (both of these codons are located in Dam

homology region I), and codon 188 [T2(Glu) vs T4(Asp)]. It should be noted that presence of an XbaI site in the T2 dam gene, but missing in T4 dam, does not alter the coding capacity. An additional complicating and startling feature is that a T2 dam subclone produces a functional hybrid polypeptide; i.e. it has a hybrid reading frame encoding the first 163 amino acids of T2 Dam followed by 84 amino acids encoded by the pUC18 vector. This hybrid enzyme methylates GATC in C-DNA, but is unable to methylate hmC-DNA, nor can it methylate all the sequences that wildtype T2 Dam recognizes in C-DNA. Although there seems to be little amino acid sequence homology between the C-terminal end provided by the vector to the missing portion of T2 Dam, they both exhibit very similar distributions of hydrophilic and hydrophobic residues.

Genetic analysis suggested that the T2 dam gene maps to a position different than where the T4 dam gene has been physically and genetically mapped in its genome. This was supported by sequence comparison of the 5' flanking regions of T2 and T4 dam genes: the two sequences start to diverge near their -35 promoter regions and on upstream. However, preliminary evidence now shows this divergence may actually be due to an approximately 600 base pair insert into the T2 genome; this insert shares no homology with T4 DNA, as shown by Southern hybridization. Homology between the 5' regions flanking the promoters of T4 dam and T2 dam resumes 5' to the inserted DNA. Finally, results of recent two-factor crosses are consistent with close linkage of the T2 dam and 56 genes, similar to the positions in T4.

REFERENCES

1. Hattman S, DNA modification: methylation, In: *Bacteriophage T4* (Eds. Mathews CK, Kutter EM, Mosig G, and Berget PB), p. 152. American Society for Microbiology Press, Washington D.C. (1983).

2. Schlagman S and Hattman S, Molecular cloning of a functional *dam*+ gene coding for T4 DNA-adenine methylase. *Gene* **22**, 139 (1983).

3. Macdonald PM and Mosig G, Regulation of a new bacteriophage T4 gene, 69, that spans an origin of DNA replication. *EMBO J* **3**, 2863 (1984).

4. Heitman J, personal communication.

5. Revel HR and Hattman S, Mutants of T2 gt with altered DNA methylase activity: relation to restriction by prophage P1. *Virology* **45**, 484 (1971).

6. Hattman S, Wilkinson J, Swinton D, Schlagman S, Macdonald PM, and Mosig G, Common evolutionary origin of the phage T4 Dam and host *Escherichia coli* Dam DNA-adenine methyltransferase genes. *J. Bacteriol.* **164**, 932 (1985).