

Mutations that Confer *de Novo* Activity upon a Maintenance Methyltransferase

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DNA methyltransferases are not only sequence specific in their action, but they also differentiate between the alternative methylation states of a target site. Some methyltransferases are equally active on either unmethylated or hemimethylated DNA and consequently function as *de novo* methyltransferases. Others are specific for hemimethylated target sequences, consistent with the postulated role of a maintenance methyltransferase in perpetuating a pattern of DNA modification. The molecular basis for the difference between *de novo* and maintenance methyltransferase activity is unknown, yet fundamental to cellular activities that are affected by different methylation states of the genome. The methyltransferase activity of the type I restriction and modification system, *EcoK*, is the only known prokaryotic methyltransferase shown to be specific for hemimethylated target sequences. We have isolated mutants of *Escherichia coli* K-12 which are able to modify unmethylated target sequences efficiently in a manner indicative of *de novo* methyltransferase activity. Consistent with this change in specificity, some mutations shift the balance between DNA restriction and modification as if both activities now compete at unmethylated targets. Two genes encode the methyltransferase and all the mutations are loosely clustered within one of them.

Keywords: protein–DNA interaction; restriction and modification; *Ral*; DNA methylation.

1. Introduction

The methylation of bases by enzymes that recognise specific nucleotide sequences within the genomes of prokaryotes plays many roles (Sternberg, 1985). Some methyltransferases are modification enzymes that protect host DNA from resident restriction endonucleases, thereby providing a host-specific imprint or identity. Other methyltransferases are not associated with restriction enzymes, for example the Dam methyltransferase of *Escherichia coli* K-12 (for a review, see Barras & Marinus, 1989). This enzyme catalyses methylation at the N-6 position of adenine within the sequence GATC, and the resulting modified DNA is an important cellular signal. The distinction between hemimethylated and fully methylated sequences provides the link between a variety of activities and the cell cycle. Methylated adenine

residues, however are not essential for *E. coli* and are uncommon in eukaryotes.

A more general modification prevalent in both pro- and eukaryotes is 5-methylcytosine, though less is known about the relevant methyltransferases and their biological roles than the Dam methyltransferase. 5-Methylcytosine is not ubiquitous in eukaryotes but where it occurs it does so primarily in the dinucleotide mCG (Adams *et al.*, 1986, 1990). The eukaryotic cytosine methyltransferases, like many prokaryotic modification enzymes, recognize short symmetrical sequences, but unlike the prokaryotic enzymes they do not methylate all potential target sequences. There is a correlation between cytosine methylation and the repression of gene activity (Cedar, 1988; Riggs, 1989) although the molecular basis for this correlation is not understood (Meehan *et al.*, 1990). Patterns of methylated residues, once established, can be maintained during DNA synthesis and cell division (Holliday, 1987; Wigler *et al.*, 1981; Stern *et al.*, 1982). One model to explain how this is achieved invokes a maintenance methyltransferase (Riggs, 1975; Holliday & Pugh, 1975) that specifically modifies hemimethylated

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