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LeTourneau University

Longview TX

Corresponding Faculty Member: Greg Frederick (GregFrederick@letu.edu)



Christina E Spencer



Katelyn Gutierrez

Identification and Implications of Soil-Dwelling Bacterial DNA Methyltransferase homologs in Mycobacterium Phage Phalm

Christina E Spencer, Katelyn Gutierrez, Frederick N Baliraine, Gregory D Frederick

Genome annotation of the temperate phage Phalm revealed two genes with homology to methylase/methyltransferase (MTases) genes in other bacteriophages, as well as in various soil-dwelling bacteria. MTases are enzymes that methylate specific bases in nucleic acids. In bacteria, MTases are known to be important to cell survival and other aspects of nucleic acid metabolism. MTases also function in epigenetic regulatory processes. Further, MTases function in restriction-modification systems. For proper cell function, nucleic acid modification is essential in directing the monitoring of the genome by other cellular enzymes. Although the functions of bacterial MTases are well known, the exact functions of MTases in bacteriophage genomes remain unelucidated.  
  
In phage Phalm and other members of the P1 subcluster, such as Brusacoram and Shipwreck, MTases genes are located next to proteins of unknown function. Similarly, in *M. abscessus subsp. bolletii strain 107*, MTase genes are surrounded by hypothetical proteins. Notably, within two or three genes on either side of the MTase genes in Phalm and other subcluster P1 phages lies a gene encoding an endonuclease or a helix-turn-helix DNA binding domain protein. Moreover, one of the hypothetical proteins in *M. abscessus* is homologous to gene 51 of phage Phalm, and in this region synteny is very similar. It is hypothesized that MTases are most likely involved in protecting the phage genomes inside their bacterial hosts. Specifically, MTases most likely protect the phage genome from restriction by host enzymes. Previous investigations indicate that MTases are necessary for stable lysogeny. Characterization of phage-encoded MTases could have relevance in host-range determination. NCBI BLASTp analysis of Phalm gp 53 and 55 align with homologs in multiple soil-dwelling bacteria. These bacteria include *Mycobacterium sp. UM\_RHS*, *M. abscessus*, *M. salmoniphilum*, *M. chelonae*, *M. fortuitum*, and *Rhodococcus*. Phalm gp 53 and 55 aligned with different site-specific MTase genes in *M. abscessus*. The presence of two MTases homologous to slightly different bacterial proteins implies that phage Phalm inhabited bacterial species possessing more than one restriction system. The acquisition and preservation of multiple MTase genes would protect subsequent generations.  
  
MTases genes in clusters N, AY, O, and AQ were also examined. The MTase genes in these phages also showed homology to multiple bacterial MTases. These gene products showed homology to bacterial MTases, specifically those of the same genus as the host species used in isolation.   
  
This study describes application of multiple bioinformatics tools, including Phamerator, NCBI BlastP, HHPred, and Splitstree, to elucidate plausible roles and significance of MTase genes in bacteriophage genomes.