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Characterization of 5-methylcytosine patterns in Pseudorabies virus

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Introduction: Pseudorabies Virus (PRV or Aujeszky's disease virus) is a neurotrophic herpes virus with a broad host range consisting of mammalian species which causes fatalities in swine populations prenatally and in young animals. It is also a widely used model organism for studies of gene expression, neuronal tracing and the viral life cycle of Alpha herpesviridae. A main characteristic of alphaherpes viruses is the strictly regulated, cascade-like gene expression pattern during lytic infection. In addition, viral genes are arranged in tightly packed functional clusters in various overlapping orientations. In order to assess whether 5-methylcytosine is present and occurs at specific loci in relation to the gene clusters in the PRV genome, wild-type and mutant PRV strains were subjected to bisulfite sequencing (BS-Seq).

Materials & Methods: PRV virions of the wild-type PRV strain Kaplan and mutant viruses lacking key trans-activators (US1, UL54, EP0 and IE180 null-mutants) were harvested 12 hours post infection from PK-15 cells by ultracentrifugation followed by DNA isolation. BS-Seq libraries were constructed for paired-end 100 bp sequencing on an Illumina Hiseq 2000. Alignments were generated using Bowtie2 following strict adapter and quality trimming; the resulting alignments were processed in Bismark for methylation calling.

Results: In PRV, interpreting CpG methylation is complicated by the extremely high GC-content of the virus. The number of methylated cytosine residues varied slightly (4-7%) between samples at lower amounts than the average methylation density in mammalian CpG islands. The intra-sample distribution of methylated residues was however non-random, indicating preferred sites of base modification and was consistent between samples which suggests that methylation at intergenic boundaries might serve important roles in viral gene expression regulation.

Biography

Peter Olah is a graduate student at the Department of Medical Biology on the University Of Szeged, Hungary. He obtained his MSc in Biotechnology from the University of Debrecen, Hungary, followed by work as a sequencing laboratory assistant. His main area is bioinformatics and sequencing data analysis related to the life cycle and gene expression regulation of herpesviruses.

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