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2024 SEA Symposium Abstract

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A Comparative Analysis of Operon Gene Functions in Cluster AZ Phages

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Operons are genes transcriptionally co-regulated by a promoter sequence. Genes in operons often encode proteins that are essential for phage proliferation such as structural and assembly genes. Bacteriophages have many genes organized into operons; however, there has been limited characterization of their structures and functions, despite being comprehensive in other models such as *E. coli*. The study aimed at identifying functions regulated by operons within the phage genome, expanding upon the role that operons play within cluster AZ phages. Furthermore, locating patterns of conserved operons between cluster AZ phages revealed which mechanisms must be organized into operons for phage survival. To identify which genes are organized into conserved operons, the genomes of representative subcluster AZ phages were compiled using GenBank and PhagesDB. Genes were denoted as likely being part of an operon if they had a nucleotide overlap of either 1 or 4 base pairs with the previous gene, indicating the overlap of the start and stop codons which would result in the continued translation of the polyprotein by the host ribosome. Clinker was used to visualize the nucleotide similarity between cluster AZ phage genomes, connecting operons with highly shared nucleotide identity with each other. 77% of our 31 identified operons were shared between AZ subcluster phages despite only an average of 50% shared gene content similarity between the subclusters. It was also found that genes necessary for the viral life cycle (i.e., structural, lysis, and replication genes) were the most common genes organized into operons. Structural and DNA replication genes occur most often as operons, each making up 34% of genes organized into operons analyzed through Clinker. Additionally, operons containing genes that perform similar functions appear in the same area of the phage genome; structural genes appear first, followed by DNA replication and nucleotide metabolism genes. The head-to-tail adaptor protein and the Cas4 exonuclease are two operon-organized gene products that were present in all phages compared. While some operons share high nucleotide sequence similarities between phages, specific genes present in these operons may differ; although there is an endolysin function present in a specific operon in the phage VroomVroom, the same shared operon in the phage Emotion has no known function instead, not having endolysin present anywhere else in the operon nor genome. This finding may also illustrate a limitation in annotation across the relatively novel AZ4 subcluster. Further research is needed to understand the mechanism by which operons are conserved between phages under diversifying evolutionary pressure.