CONSIDER FOR TALK

2024 SEA Faculty Meeting Abstract

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Comparative sequence and structural analysis reveal cyclic oligonucleotide sequestering immune evasion proteins in Actinobacteriophage genomes

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The production of cyclic oligonucleotides in response to innate immune sensing of viral infection is conserved throughout all domains of life. After production, cyclic oligonucleotides act as secondary messengers that activate antiviral signaling pathways resulting in the expression of antiviral genes, autophagy, or cell death. In bacteria the nucleotidyl-transferase enzymes that enable the production of cyclic oligonucleotides are typically found in operons known as cyclic-oligonucleotide-based antiphage signaling systems (CBASS). Bacteriophages have evolved to inhibit CBASS signaling systems by expressing anti-CBASS nucleases (Acb1) that degrade cyclic oligonucleotides and prevent the activation of downstream effectors. Work from the past two years has also revealed that bacteriophages can evade this pathway by expressing cyclic oligonucleotide sequestering proteins (Acb2). The crystal structures of two of these proteins showed that they form hexamers that can bind three, di or two, tricyclic nucleotides via distinct binding sites. These published structures enabled the discovery of homologues in Actinobacteriophage genomes Balomoji(E) and Wolfstar(ED) during genome annotation this year. Examination of multiple sequence alignments of these sequences and their published homologs reveals variability in the conservation of residues required for di- and/or tri-cyclic nucleotide binding. High confidence AlphaFold-Multimer models of Actinobacteriophage homologues allowed the 3-D visualization of conserved residues compared to published structures and provides additional support for cyclic oligonucleotide binding activities. The predicted spectrum of cyclic oligonucleotide binding activities will be described from three different Actinobacteriophage phams, to support a proposal to add these functions to the SEA official phage protein function list. Finally, we will share tips for other phage instructors to work with students to identify new protein functions.