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2023 SEA Symposium Abstract

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Validation of the 3-Dimensional Structure of Cluster A1 Mycobacteriophage Adahisdi’s Repressor Protein Using Alanine Scanning

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Upon infection of a host cell temperate bacteriophages can enter into either the lytic or lysogenic cycles. During the lysogenic cycle the bacteriophage enters into a long-term relationship with the host cell. The bacteriophage genome becomes a part of the bacterial genome and is copied along with that of the host as the cell divides. To prevent entry into the lytic replication cycle bacteriophages often express a repressor protein. Repressor proteins “repress” the transcription of viral genes by binding to specific sequences found within the bacteriophage’s genome and physically block transcription of genes in this area. Though the majority of Mycobacteriophages are temperate few repressor genes have been biochemically characterized, and only one 3-dimensional structure has been reported for a cluster A2 bacteriophage TipsytheTRex. Cluster A is the largest cluster and contains 20 subclusters. Superinfection immunity phenotypes within this cluster are complex exhibiting a spectrum of homo to complete hetero-immunity. Cluster A2 and cluster A1 bacteriophages are hetero-immune and express repressor proteins that are known to bind to different DNA consensus sequences. The cluster A1 bacteriophage Adahisdi repressor protein is 170 amino acids in length and contains two DNA binding domains. The repressor protein is known to bind to a 13mer DNA consensus sequence (CTTGATTCGTAAC) that appears many times throughout the Adahisdi genome. The research of the Spring 2022 and 2023 Biochemistry laboratory classes has focused on validating the 3-dimensional structure of the Adahisdi repressor protein bound to DNA. Specifically, our work has centered around mutating the side chains of amino acids that interact directly with DNA bases to match that of the amino acid Alanine. Plasmid constructs were created that express mutant versions of the Adahsidi repressor protein via a combination of Site-Directed mutagenesis and HiFi-Assembly. We will describe the impact of these mutations on the ability of the Adahisdi repressor protein to prevent lytic viral replication in vivo and bind DNA in vitro. Similarities and differences to the cluster A2 repressor structure will be discussed.