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Identifying Novel Characteristics of Methyltransferase and Hydrolase Structures in Phage Tinybot Genome

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In this project, we study the characteristics of two genes chosen from the newly sequenced Mycobactriophage Tinybot. Eighty three genes were predicted to be encoded in its 51,402-bp genome. Based on restriction digestion and sequence analysis, it is assigned to A4 subcluster. We have chosen two genes (DNAM78 and DNAM80) for further bioinformatic analysis. According to our analysis, DNAM78 and DNAM80 likely belong the larger methyltransferase, and hydrolase family, respectively. Methyltransferase is part of a viral restriction modification system where, prior to insertion into the host cell DNA, the phage genome is methylated to avoid DNA degradation caused by restriction enzymes in the host cell. Ultimately, it allows the phage to integrate its genetic material into the host genome. Hydrolase degrades peptidoglycan present in the host’s cell membrance, permitting injection of viral genetic material into the host cell. Goals of this project include (i) confirming predicted protein functions called by DNA Master, BLASTP, and HHPred, (ii) comparing protein structures predicted by Protein Data Bank (PDB), I-TASSER, and PredictProtein, and (iii) determining the relationship between structure and function of the two proteins. We used a multiple sequence alignment program CLUSTAL Omega to align DNAM78 and its homologous proteins. Result indicated several highly conserved regions in which they may serve important biological functions. Conserved regions were mapped to the predicted protein structure, suggesting these residues have large potential for ligand binding activity. For hydrolase DNAM80, the homologous protein structure found in PDB and the predicted structure produced by I-TASSER share a common extended C-terminal strand, with a putative protein-binding function at amino acid 154. We speculate that the extended tail undergoes conformation change when it interacts with the cell membrane, exposing an internal active site for ligand binding. In conclusion, we combined experimental and bioinformatic methods in elucidating the function and the structure of two novel phage proteins. Our findings shed light on their roles in infection. Further experiment is needed to characterize their biochemical and cellular functions.