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Unveiling Bromden's Secrets: A Nicotinate Ribosyltransferase as the Ultimate Power Play Gene

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With an estimated population of 10^31 individuals, bacteriophages are the most abundant and diverse form of life present on this planet. This diversity can be observed among the collection of Actinobacteriophage phamilies (families of genes): over 70% of annotated genes have no known function (NKF).

Following the success of the analysis of the Fruitloop and Waterfoul phage genomes, the University of Ottawa joined the SEA-GENES adventure with the gene characterization of the mycobacteriophage Bromden. As part of the poorly characterized L4 cluster, Bromden contains 118 genes, of which 67 (57%) are NKF, and 13 tRNAs.

Each of Bromden's genes was cloned into the pExTra inducible plasmid and overexpressed directly in Bromden’s host, *Mycobacterium smegmatis mc2155*. The library has been sequenced, verified and sent to HHMI. We analyzed two phenotypic characteristics, cytotoxicity and immunity to Bromden and related phages, in order to gain clues about gene function.

During our analysis, we found that 34% (40 genes) of Bromden genes are linked cytotoxicity, ranging from slight morphology changes (gp24, NKF) to >3-log reduction in the number of colonies formed (gp62, NKF). Out of those, 28 genes were reproducibly impacting the growth of *M. smegmatis*.

Notably, two genes lead to the complete death of the host: gp75 (nicotinate Ribosyltranferase) and gp127 (NKF) prior to the induction of the plasmid. We experimentally demonstrated that these genes were able to escape the control of the inducible promoter. Using AlphaFold2 and Dali, we found a match to gp75 with a human protein, Nicotinamide Phosphoribosyltransferase. This protein is used to elevate cellular level of NAD+, one of the main energy currencies in cells. Using key residues in the active site of the human protein we created three point mutations in gp75, and the S276A mutant was transformable, suggesting that activity of this enzyme is responsible for its potent toxicity.

We have encountered difficulties testing immunity after gene overexpression because Bromden infects its host poorly at 37˚C, the temperature used for gene induction. Although Bromden infection is effective at killing its host at 30-35˚, we have had inconsistent immunity results at these temperatures when we are also selecting for the overexpression plasmid and inducing gene expression.

This is a first step to uncover phage gene function. Future work will focus on the protein-protein interactions and gene deletion to characterize further the gene function. Understanding the mechanisms phages use to kill their hosts and that hosts use to defend themselves against phages are essential to developing new therapeutic targets that maximize bacterial killing and limits bacterial defense mechanisms.