CONSIDER FOR TALK

10th Annual SEA Symposium Abstract

Howard Hughes Medical Institute

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Gene Detectives: Exploring Phage Gene Function

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In the past decade, the SEA-PHAGES program has isolated and characterized thousands of phages, providing a wealth of genetic data to the broader scientific community. These data have already furthered our understanding of the diversity and evolutionary history of phage populations; they have also revealed the vast unknown contained within phage genomes. In their 2015 comparative genomic analysis, Pope et al. reported that out of the 5,205 gene phamilies found in 627 mycobacteriophage genomes, approximately 75% have no assignable function. Characterizing the functions of these phage genes is an important endeavor that will provide greater insight into phage-host and phage-phage dynamics and potentially inspire advances in therapeutics and molecular technologies.

The SEA is currently developing research tools and methods that will allow the SEA community to utilize this expanse of genetic data and investigate phage gene functions. These include:

1) A workflow for the construction of phage gene expression libraries that can be utilized in various bacterial host expression assays. This workflow was successfully implemented as "pilot projects" at three undergraduate institutions, generating four phage gene libraries that were then systematically analyzed to identify a subset of genes that inhibit *M. smegmatis* growth.

2) A bacterial two-hybrid selection platform that allows for the rapid testing of over a million pairwise interactions between a phage protein of interest and a library of *M. smegmatis* protein fragments, thus enabling the identification of putative phage-host interactions using straightforward yet powerful genetic techniques.

Part of this workflow was successfully implemented as "pilot projects" at three undergraduate institutions, generating four phage gene libraries that were then systematically analyzed to identify a subset of genes that inhibit *M. smegmatis* growth. Together, these phenotypic and interactome analyses can open the door to new hypotheses and mechanistic questions for the community to explore. We believe that by collecting evidence piece-by-piece, the SEA can be an important driver in the elucidation of phage gene function.