

2_2 ModelPhagesAndTheirProperties_Blasdel

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Slide 1: The Vision for Model Phages

In the 1930s, inspired by Erwin Schrodinger's influential book *What is life*, Max Delbruck developed a vision for bacteriophage as a living system simple enough to truly understand on a fundamental level, even with the rudimentary tools his Phage Group had available to it. They reasoned that, if we were ever going to understand how life works, we would need to start with the simplest organism possible and work our way up. He selected and renamed seven obligately lytic bacteriophages against *Escherichia coli* B, and organized them into a series labeled T1 through T7. The central idea was that he and his growing number of colleagues would focus on truly understanding how these phages worked and use that knowledge to generalize to *E. coli*, then the mouse, and then us.

Slide 2: What Was Accomplished

This vision of bacteriophage, along with the elegant work of early bacteriophage biologists, quickly provided a mechanistic foundation that radically reoriented our understanding of life on Earth. Taking advantage of the simplicity of bacteriophage, they described mutations as the units that natural selection acts upon, established DNA as the heritable molecule, first described the triplicate nature of codons in mRNA, and elucidated Central Dogma. Indeed, it was this elegant simplicity of the bacteriophage lytic infection cycle on a microbiological level that allowed Luria and Delbrück to initially demonstrate that genetic mutations arise in the absence of selection rather than being a response to selection. Similarly, it was the elegant simplicity of bacteriophage viral particle chemistry with just two types of molecules, which could be differentially radiolabelled, that allowed Hershey and Chase to demonstrate once and for all that DNA is the heritable molecule.

Following up on these efforts, much of the work that underlies the molecular basis for bacterial physiology, biochemistry, genetics, genomics, and then recombinant engineering was made possible by the elegantly determinative experiments that can be performed because of how completely phage systems can be understood. Indeed, many of the molecular tools we take for granted today, from T4 DNA ligase to the T7 expression system, are derived from phage. With the extraordinary power now made available by next generation “-omics” techniques, Delbruck's historical vision of phage as the underlying model organism of molecular biology has the chance to gain new relevance.

Slide 3: Bacteriophage T4

Studies of Phage T4 and its relatives have provided countless contributions to our understanding of molecular genetics and biochemistry. Indeed, it was used as the model organism for many of the discoveries I have just mentioned. A lot of its value stems from how it completely substitutes Hydroxymethylcytosine for Cytosine (The “C” in the four letters of the DNA code) and then glucosylates it in such a way as to make its own DNA chemically distinct from its hosts'. This chemical distinction

allows phage T4 to completely shut off the expression of host genes, ensuring that that only phage genes will be expressed.

Slide 4: Bacteriophage T7

The T7 phage and its relatives are in many ways most notable for the elegant simplicity of their transcriptional scheme as well as the extraordinary usefulness of the RNA polymerase it uses for it. Upon entering the cell, T7 recruits the host transcriptional apparatus with strong sigma70 promoters to transcribe early genes, including its own single subunit RNA polymerase, before inactivating the host RNA polymerase. The Phage RNA polymerase then transcribes middle/late genes from phage-specific promoters that replicate the phage genome and construct infectious viral particles. The parallel transcriptional system that T7 uses to continue expressing its own middle/late genes while shutting off host expression is the hallmark of the *Autographvirinae* subfamily (the source of its name) and now one of the core tools of molecular biology. Indeed anytime a researcher wants to express a given DNA sequence without using mechanisms found in the host that could have unpredictable side-effects, that sequence can be controlled by a T7 promoter that is only recognized by the T7 RNA polymerase.

Slide 5: Bacteriophage N4

Enterobacteriophage N4 infection progresses by sequentially expressing phage genes using three distinct RNA polymerases, including one virion associated RNA polymerase (vRNAP) packaged into the capsid along with its DNA. Phage N4 infection begins when the phage injects its vRNAP as well as a short sequence of DNA into the cell, which is then transcribed in the forward direction using host factors and the vRNAP, both creating early transcripts and pulling the rest of the genome into the cell. The second virally encoded RNA polymerase (N4 RNAPII, gp15-16), as well as two virally encoded transcription factors (gp01-02), are then expressed from these early transcripts and together promote the middle mode of transcription involving gene features associated with DNA replication. This includes one single stranded DNA binding protein that interacts with the host sigma70 sigma factor to promote late phage transcription of structural and lysis genes.

Slide 6: Taxonomic Context

It is important to keep in mind that learning about these model phages does not just give us important information about the structure and function of living systems generally, as their simplicity allows us to isolate and truly understand those systems, but also give us meaningful information about how they and their relatives function. Indeed each of these three clades of phages are currently being considered for therapeutic applications addressing a variety of important Gram negative pathogens, and each have significant environmental impacts worth understanding.