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A century of phage research: Bacteriophages and the shaping of modern biology

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The dawn of phage research

One hundred years ago, in 1915, the science of virology was in its infancy, and Frederick Twort was having problems with his cultures. Twort, an English physician then aged 37, was attempting – unsuccessfully – to propagate vaccinia virus, the primary component of the smallpox vaccine, on agar plates. Rather than vaccinia, however, the only things growing on Twort's plates were contaminating bacteria.

Or so it seemed at first. Despite the lack of progress towards his original goal, Twort soon observed that something else was happening: mysterious "glassy and transparent" spots, which turned out, upon closer examination, to be zones of dead bacteria, would occasionally materialize on his plates. Moreover, these zones were transmissible – clear spots begat clear spots, even when greatly diluted – and specific to the type of bacteria that were contaminating his plates. Twort, not one prone to speculation, proposed three possible explanations for this strange phenomenon: it could be an unusual manifestation of the bacterial life cycle, or an enzyme produced by the bacteria themselves or, most radically, some sort of "ultra-microscopic virus". Unable to confirm or reject any of these hypotheses with the resources at his disposal, Twort wrote up his findings [1] and let the matter drop.

Little could Twort imagine how quickly things would change – or how important his "transparent material" would ultimately become. Two years later, the outspoken French-Canadian microbiologist Felix d'Herelle independently published similar observations [2], but unlike the more cautious Twort, d'Herelle was immediately convinced that he had discovered a new type of virus that infected bacteria, which he dubbed a bacteriophage (phage). D'Herelle's contention was eventually validated, and much more besides: over the

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Conflict of interest

The author declares that no conflict of interest exists.

next century, phages would be identified as some of the planet's most influential biological entities and would play a leading role in some of biology's most meaningful advances.

Phages and their biology

For most of this first century of phage research, phages' ecological and environmental significance attracted little attention. Over the last 25 years or so, however, it has become clear that phages are the most abundant organisms on Earth. There are an estimated 10^{31} phage particles on the planet [3], an impossibly large number that translates into approximately a trillion phages for every grain of sand in the world. Phages have been isolated from every environment in which bacteria exist, and it is believed that at least one type of phage – and most likely many more than one – infects every strain of bacteria. And our current understanding represents just the tip of the iceberg: there are fewer than 1,500 complete phage genomes listed in GenBank (as of August 2014), many of which have not been studied experimentally, but there are thought to be many tens of millions of phage species in natural environments.

Another striking feature of the prokaryotic virosphere is its remarkable diversity. Phage virions vary widely in size, shape and complexity, and the genomes they contain are, if anything, more diverse still. Phage genomes range in size from 3.4 kb to almost 500 kb, and unlike bacteria, there is no single gene (e.g. 16S rRNA) present in all phage genomes. In fact, phage genomes are highly mosaic: like words forming sentences, each unique genome represents a different combination of the same modules that have been shuffled and re-shuffled over billions of years of viral evolution. Indeed, phage genomes likely represent the largest reservoir of unexplored genes and proteins anywhere in the biosphere.

Given their ubiquity and diversity, it is not surprising that phages play profound roles in a variety of biological and environmental processes. It is estimated that phages kill and lyse between 15% and 40% of the ocean's bacteria every day, thereby influencing the ratio of particulate to dissolved carbon, rates of phytoplankton productivity and oxygen production, and perhaps even global climate and weather patterns [4]. Moreover, phages are significant drivers of bacterial evolution, both because bacteria must constantly evolve to avoid being killed by their viral predators and because phages, especially temperate phages (those that can stably integrate their own genome into the genome of their host), are prominent agents of horizontal gene transfer. Globally, it is believed that phages mediate gene transfer events between bacteria (transduction) up to 20 million billion times per second [5].

Some phages' adeptness at moving genes around can also pose a significant threat to public health. Like Dr. Jekyll and Mr. Hyde, free-living or commensal bacteria that constitute little risk to human health can be rapidly converted by certain temperate phages into aggressively virulent pathogens. Many phage-free strains of *Escherichia*, *Salmonella*, *Staphylococcus*, *Streptococcus*, and *Vibrio*, among others, are not normally considered dangerous, but they can become deadly by acquiring just a few virulence factors, usually exotoxins or enzymes, upon infection by specific temperate phages bearing those genes.

Other phages affect health in more positive ways. For example, phages colonize the mucosal surfaces of humans and other animals, where they reduce bacterial colonization and

essentially act as an additional component of innate immunity [6]. Phages, especially lytic phages (those which always kill, rather than occasionally integrate with, their hosts), also have considerable therapeutic potential. Nearly 100 years ago, d'Herelle envisioned a future in which phage therapy, or the use of phages to kill pathogenic bacteria, would play a leading role in combating infectious diseases, and although the emergence of cheap and effective antibiotics led to the abandonment of phage-based medicine in the West, the rise of antibiotic resistance has led to renewed interest in phage therapy. Today, a century after their discovery, phages are poised to fulfill their early promise and make a significant contribution to the treatment of bacterial disease.

Phages and our study of biology

Even as phage therapy began to be displaced by chemotherapeutics, phages did not entirely fade from view, thanks largely to their emergence as model organisms. In the late 1930s, a postdoctoral researcher at Caltech, Emory Ellis, began using phages to investigate basic viral biology, which he hoped would shed light on the role of viruses in cancer. Around the same time, a German physicist-turned-geneticist, Max Delbrück, arrived at Caltech with an interest in establishing the physical nature of the gene. Although originally intending to further that goal by working with T.H. Morgan and *Drosophila*, Delbrück encountered Ellis and, impressed by phages' convenience and simplicity, quickly abandoned flies for viruses.

It was to prove a critical decision. Although Ellis soon returned to cancer research, Delbrück continued his work on phage replication, and in the early 1940s, he began an informal collaboration with two other researchers, Salvador Luria and Alfred Hershey. Over the next twenty or so years, Delbrück, Luria, Hershey, and their students and colleagues came to constitute what would become known as the Phage Group, a diverse set of researchers using the same few model organisms (the lytic T-series phages of *E. coli*) to probe, in great detail, the molecular workings of inheritance and reproduction which enabled a humble phage to reproduce itself a hundredfold in less than 30 minutes. Other researchers, including Francois Jacob and Esther Lederberg, used temperate phages (most notably, λ) to explore the intricacies of gene regulation and the ways in which genotype and environment come together to influence behavior – even at the edge of life.

Ultimately, the legacy of these early phage workers lies in their role in helping to transform biology into a modern science, one capable of studying the mysteries of life in unprecedented detail. Their long-term commitment to the same small group of model organisms meant that phages were used in many of the key experiments which elucidated fundamentals of biology we take for granted today. Some of the most important of these phage-enabled findings are summarized in Table 1. Moreover, and perhaps more importantly, the phage trinity's mentorship, centered around an annual summer phage course at Cold Spring Harbor Laboratory, instilled in their disciples (which included James Watson, Frank Stahl, Renato Dulbecco, and numerous other pioneers in molecular biology, genetics, and virology) a rigorous and consistent set of standards for experimental design, deductive reasoning, and data evaluation that had, until then, been lacking. In several ways, then, phages helped to make possible an end result which was, as the Nobel laureate John

Kendrew put it, the “most dramatic and rapid development in the whole of biology since Darwin and Mendel [7].”

Phages have also featured prominently in more recent scientific breakthroughs. The first gene (phage MS2 coat protein; 1972), RNA genome (phage MS2; 1976), and DNA genome (phage φX174; 1977) to be completely sequenced were all phage-related. Phage display, first introduced in 1985, is a technique in which libraries of proteins or peptides are fused to phage particles and screened for biological relevance. Since a phage culture can contain billions of viral particles, each expressing a different protein, phage display is enormously high-throughput, and by facilitating the isolation of monoclonal antibodies and receptor-binding ligands of virtually any specificity, phages have helped to establish pharmaceutical niches worth tens of billions of dollars. Most recently, studies of bacterial phage-resistance mechanisms led to the discovery of the CRISPR/Cas system, a prokaryotic version of adaptive immunity in which bacteria retain genetic “memory” of past phage encounters (CRISPR loci) and produce complementary small RNAs that direct Cas9 nucleases to cleave that same phage DNA should it be encountered again. Cas9 activity is not limited to phage DNA, and by utilizing different guide RNAs complementary to different genes of interest, CRISPR-based techniques are rapidly becoming the method of choice for targeted mutagenesis and genome editing in everything from yeast to plants to mice. Seventy-five years after Delbrück and colleagues used phages to launch a revolution in biology, phages continue to shape the way we do science today.

Phages and the future

Given the insights gained from and about phages over the past century, what might the next 100 years of phage research entail? Although a short essay cannot do justice to the ambitions of a multifaceted and rapidly expanding field, it can put forward, in broad terms, some plausible predictions of things to come.

- A variety of phage-based therapies will become widely used in medicine and agriculture, especially in the context of drug-resistant and/or long-term bacterial infections. Encouraging data from clinical trials will continue to accumulate, current regulatory hurdles will be overcome, and therapeutic phages’ utility will be enhanced (degrading microbial biofilms; disrupting bacterial signaling pathways, etc.) through genetic engineering and synthetic biology [8]. Moreover, phages will be increasingly used as platforms for drug delivery and antigen display, in pathogen detection, and as vaccines.
- The human body’s abundance of phages [9] and other viruses will be recognized as a critical mediator of human health and well-being. Over the last 20 years, it has become increasingly clear that our communities of resident bacteria greatly influence our health, nutrition, and even behavior; over the next 100, we will learn that our phage communities are similarly important, thanks both to their impact on those same bacteria and their mediation of horizontal gene transfer.
- Insights derived from studies of phage ecology and evolution (in both model and natural systems) will lead to a better understanding of viral emergence and to new

strategies for combating viral pathogens. By considering viruses' natural environments, ecological niches, and life history strategies – in other words, by viewing them as living organisms in a broader biological context – we will be better able to anticipate future threats and develop innovative approaches (e.g. ecological traps [10]) for countering current and future pathogens.

- Phage genomes and proteomes will be extensively mined for novel genes and proteins. Because phages are incredibly ancient, numerous, diverse, and on a global scale uncharacterized, continued advances in metagenomics will allow us to identify novel phage products suitable for applications in medicine, agriculture, biotechnology, bioremediation, energy generation, and other such fields.

Over the next century, yet-to-be-discovered phages and phage products, along with those already known to science, will allow us to ask new questions, solve new (and old) problems, and achieve new milestones in biology. A hundred years after Frederick Twort noticed strangely clear spots on his plates, phages will still be shaping our world and the ways in which we study it.

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Table 1

Major biological discoveries involving phage, 1940–1970. Asterisk (*) denotes Nobel laureate.

Year	Researcher(s)	Finding(s)
1943	S. Luria*, M. Delbrück*	Mutation is spontaneous and random (pre-adaptive), not directed (post-adaptive)
1945	S. Luria*	Viruses can mutate so as to be able to successfully infect previously resistant bacteria
1946	A. Hershey*, M. Delbrück*	Two viruses can genetically recombine when co-infecting the same cell
1950	A. Lwoff*, L. Siminovitch, N. Kjeldgaard	Ultraviolet radiation can induce the excision of a viral genome from that of its host
1951	V. Freeman	Viruses can contribute to bacterial virulence (*e.g.*, by encoding diphtheria toxin)
1952	A. Hershey*, M. Chase	DNA, not protein, is unambiguously the hereditary material of life
1952	N. Zinder, J. Lederberg*	Viruses can transfer DNA between cells (transduction)
1952	S. Luria*, M. Human	Once adapted to a particular host, viruses' ability to infect other hosts is greatly diminished (this "restriction" was later shown to result from restriction enzymes)
1955	S. Benzer	The sequence of a gene is linear; recombination can occur between adjacent nucleotides
1961	B. Hall, S. Spiegelman	Complementary DNA and RNA can hybridize
1961	F. Crick*, L. Barnett, S. Brenner*, R. Watts-Tobin	Nucleotides are read three units at a time (as codons) to form proteins
1961	S. Brenner*, F. Jacob*, M. Meselson	mRNA is the intermediate between DNA and protein; mRNA is translated into protein by ribosomes
1962	D. Nathans*, G. Notani, J. Schwartz, N. Zinder	Purified RNA can direct the synthesis of its encoded protein in a cell-free environment
1967	M. Gellert (and other laboratories)	DNA ligase joins together DNA fragments
1967	M. Goulian, A. Kornberg*, R. Sinsheimer	DNA can be synthesized from its precursors *in vitro*
1969	R. Okazaki, T. Okazaki, K. Sakabe, K. Sugimoto, A. Sugino	Lagging strand DNA synthesis is discontinuous