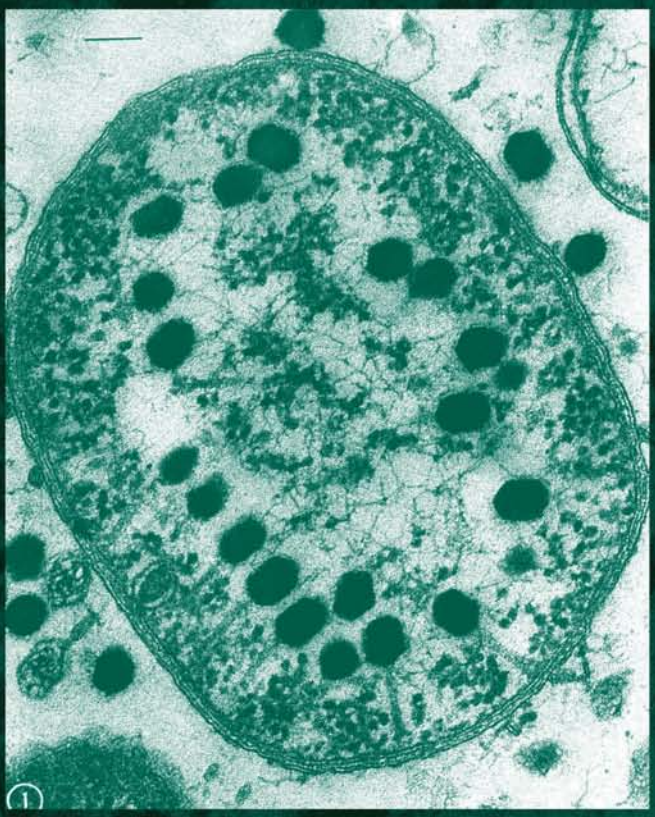
BACTERIOPHAGES

Biology and Applications



EDITED BY

Elizabeth Kutter

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Foreword

It is a privilege for me to have this opportunity to provide a brief foreword to *Bacteriophages: Biology and Applications* by Elizabeth Kutter and Alexander Sulakvelidze. I was one of many who first became fascinated with the romance of science by reading the book *Arrowsmith* as a teenager. In that novel written by Sinclair Lewis in 1925, an attempt to develop phage therapies against bacterial diseases played a central role. But by the early 1950s, when I read the book, the widespread success of newly introduced antibiotics had seemed to make this alternative approach to the selective killing of bacteria unnecessary.

Instead, a small set of bacteriophages had begun to attract attention as "model organisms"—prime systems for probing the basic chemistry of life. These phages were attractive to scientists, because they were much easier to study with the then-available tools than were more complex life forms such as bacterial or human cells. They had relatively small genomes and multiplied rapidly, making them unusually amenable to genetic analyses that aimed at obtaining multiple mutants in each bacteriophage gene. To enable the essential genes for viral multiplication to be genetically identified, screening techniques were developed that focused on *conditional lethal* mutations—for example, through the identification of "temperature-sensitive" phage mutants that would grow at low—but not high—temperatures. Moreover, because large amounts of infected cells were easy and inexpensive to obtain, biochemical approaches could be readily employed, so that the products of the genes identified by genetic screens could be isolated and characterized in cell-free systems.

The model organism approach worked better than anyone had had a right to expect, in part because the mechanisms that are used to control gene expression and to recombine and replicate DNA genomes turned out to be much more highly conserved across life forms than anyone had suspected. Much of the work was concentrated on several viruses that infect the bacterium *E. coli*—most notably the bacteriophages lambda, T4 and T7. The findings made in multiple laboratories could thereby be combined, yielding results that were immensely important in developing the field of molecular biology, as reviewed in the early chapters of this book.

To give a personal example, for 30 years beginning in 1965, my own laboratory would exploit the combined genetic and biochemical advantages of the T4 virus for study of fundamental DNA replication mechanisms. In the end, the "protein machine" mechanisms revealed at the replication fork through bacteriophage studies turned out to be highly similar to those used to move the replication forks of higher organisms, including those of humans (Alberts, 2003).

In the 1960s and 1970s, many advances were made in a wide range of laboratories studying both bacteriophages and the bacterial cells themselves. The new knowledge of biological mechanisms that resulted soon allowed the development of more powerful research tools (such as DNA cloning). With these new tools, researchers could begin to unravel the molecular mechanisms in more complex cells and organisms.

As a result, by the 1980s most of the action and excitement in molecular biology had moved away from simpler organisms to investigations of mammalian cells.

For several unrelated reasons, we may have come full circle over the course of the last 80 years. First of all, there is an urgent need for new types of antibacterial therapies. We now live in an ever-more crowded, more interconnected world in which resistant strains of microorganisms spread with amazing rapidity. Modern science has increased our ability to design countermeasures to these diseases of humans and animals. The standard countermeasures have been new drug and vaccine developments. But producing a new drug is an enormously expensive endeavor. In addition, market failures have discouraged the development of new vaccines in the private sector. As a result, the world now faces a serious challenge in dealing with a host of microbial threats that were once thought to be defeated rather easily by antibiotics (Institute of Medicine, 2003). As described in Chapters 12 to 14, there is therefore every reason to reintroduce bacteriophage therapies as an additional tool in the war against bacterial diseases.

A second feature of modern biology that is reawakening interest in bacteriophages is our new ability to obtain the DNA sequences of large numbers of organisms inexpensively. From this DNA sequence information, we can determine the relatedness of organisms and attempt to retrace the past history of life on the Earth. The sequencing of bacteriophages is only just beginning. Not only are there immense numbers of novel proteins yet to be discovered among what could be 100 million different bacteriophages in the environment—the vast majority not yet known (the genomes of only about 400 have thus far been completely sequenced)—but it is now suspected that some of the lytic phages carry genes that trace back in evolutionary history to the common ancestor of eukaryotic and prokaryotic cells (see Chapter 5). In summary, bacteriophages represent a huge untapped genetic reservoir that can be productively mined—both by those interested in proteomics and by those who are trying to decipher the mysterious nature of the early cells that predated the split among the three families of cells that are alive today: the archaea, the bacteria, and eukaryotes.

Now that we have access to the complete molecular anatomy of a cell, a third reason for a new focus on bacteriophages stems from the realization—sobering to scientists like myself—that biological systems are so complex that they cannot be understood without new methods of analyzing and conceptualizing them. Thus, for example, the nearly 500 different protein molecules that are encoded by the genome of the simplest known living cell, the small bacterium *Mycoplasma genitalium,* interact with each other and with substrates in an enormous number of ways. Even if we had a complete catalog of all of these interactions and their rate constants, information we are far from achieving today, we could not claim to understand this cell in any deep sense—that is, in the sense of being able to explain how the cell is able to grow and reproduce itself as a chemical system. Living systems are made possible by a huge web of networked chemical reactions, and we presently lack the tools to decipher what is most significant within such complexity. This realization, new to most molecular biologists, raises the question of whether it might be productive to focus once again on one or a few bacterial viruses that could serve as model organisms—far simpler than any free-living cell—for developing new types

of complexity analyses. If so, which viruses should be targeted and through what types of experimental strategies?

Finally, the increasingly large role that science and technology will play in driving societal changes in the twenty-first century argues strongly for a new type of science education in our schools. Beginning with 5-year-olds, what is needed is an education that allows students to explore the world around them using evidence and logic, so that they leave school learning to solve problems the way that scientists do. They also need to understand what science is and why it represents a special way of knowing about the natural world, if they are to respect its judgments concerning the many important issues that they will need to decide in their lifetimes—such as whether they should avoid exposures to substances that could adversely affect their health in the future, or whether their nation should make sacrifices to reduce the release of greenhouse gases into the atmosphere.

The National Science Education Standards call for a revolutionary change in science teaching, with an emphasis on teaching science as inquiry (National Research Council, 1996). As the ultimate step in such an education effort, it should be possible for a select group of students to participate in a real scientific investigation in their upper years of high school. It is thus encouraging to find high school students appearing as coauthors of a major publication from the University of Pittsburgh, in which a diverse set of novel bacteriophages that infect mycobacteria have been identified and sequenced (Pedulla et al, 2003).

The National Academy of Sciences has just published the results of an unusual workshop in which 25 leading scientists outside the field were exposed to the biology of the smallpox virus and challenged with the task of suggesting new approaches to antiviral therapies (Harrison et al, 2004). As this exercise made clear, we badly need a new infusion of talent and energy into the field of virology, where there is an enormous opportunity for scientific breakthroughs whose results will be of great practical benefit to human health (Alberts and Fineberg, 2004). What better way to recruit outstanding young people into such fields than to expose them as teenagers to a scientific exploration of the wonderfully rich and diverse world of bacteriophages?

I would like to end by congratulating both the editors and the many contributors to this volume for their dogged persistence in sticking to bacteriophage research over many decades. They have survived their years in the shadows, and now we can all appreciate the strong platform that their work has established for the many exciting years of research ahead.

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Editors

Elizabeth Kutter, Ph.D. has been a member of the faculty in biophysics and head of the laboratory of phage biology at The Evergreen State College, Olympia, WA, since 1972. She received her B.S. in mathematics at the University of Washington, Seattle, in 1962 and her Ph.D. in biophysics at the University of Rochester, New York, in 1968. Her thesis, under John Wiberg, dealt with bacteriophage T4's substitution of hydroxymethylcytosine for cytosine in its DNA and the transition from bacterial to phage metabolism, and her research has focused on phage biology ever since. From 1969 to 1972, she worked with Rolf Benzinger at the University of Virginia. She took the NIH grant she won there to Evergreen, where she has maintained an active undergraduate research program, funded largely by the National Science Foundation (NSF) and National Institutes of Health (NIH). From 1975–1980, she was a member of the NIH Recombinant DNA Advisory Committee, drafting the initial national guidelines in the field. She has also served on many grant and oversight panels at NSF, NIH, and the Howard Hughes Foundation and as a reviewer for various journals.

From the time of her first talk in 1964 at a Cold Spring Harbor phage meeting, Dr. Kutter was drawn by the highly collaborative nature of the phage field. In 1975 she started holding West Coast T4 Meetings which developed into the biennial Evergreen International Phage Biology meetings; the 15th, in 2003, drew 100 scientists from 14 countries. She spent 1978–1979 as a sabbatical fellow with Bruce Alberts at University of California at San Francisco, where she began work on the T4 genome project—the start of a worldwide collaboration which included 4 months in 1990 in Moscow, Pushchino, Vilnius, and Tbilisi through an exchange program between the U.S. and Soviet Academies of Science. During this visit, she also learned about the extensive Soviet history of phage use as antibiotics. Her initial skepticism about phage therapy gave way over the next few years as she talked and worked with researchers, physicians, and patients in Tbilisi, Republic of Georgia, where it is a standard part of care for purulent and gastrointestinal infections. In 1997, she and Evergreen colleagues established the nonprofit PhageBiotics Foundation to help stimulate worldwide interest in exploring the possibilities and challenges of phage therapy, including the broad studies of phage biology crucial to this endeavor.

Dr. Kutter was an editor and author of *Bacteriophage T4* (ASM, 1983) and *The Molecular Biology of Bacteriophage T4* (ASM, 1994), which grew out of the Evergreen T4 meetings, and of *The Encyclopedia of Genetics* (Academic Press, 2002). She has also co-authored dozens of other papers, book chapters, and popular articles, as well as over 100 meeting presentations related to phage, and participated in programs on CBS's *48 Hours, Dateline Australia, Discover-Canada, NPR Science Friday,* and *Voice of America*. This current book is a natural outgrowth of these various aspects of her work, which has included studies of the molecular mechanisms of host DNA degradation, nucleotide biosynthesis and transcription regulation after

phage infection and of phage ecology, genomics and evolution, and has particularly focused on phage as an effective teaching tool.

Alexander Sulakvelidze, Ph.D. is an associate professor of epidemiology and preventive medicine at the University of Maryland School of Medicine, and vice president of research and development and chief scientist of Intralytix, Inc. Dr. Sulakvelidze received his formal training in microbiology in the former Soviet Union, including a B.A. from Tbilisi State University in 1986 and a Ph.D. in microbiology and epidemiology from Tbilisi State Medical University in 1993. He continued his training at the Engelhard Institute of Molecular Biology in Moscow, and, under the auspices of the U.S. National Academy of Sciences, at the University of Maryland School of Medicine in Baltimore.

Dr. Sulakvelidze's research interests are in the broad areas of emerging infectious diseases, molecular epidemiology, pathogenesis of bacterial enteric diseases, and phage therapy. A major focus of his research involves studies of the potential usefulness of bacteriophages in preventing and treating infectious diseases caused by multi-drug-resistant bacteria. In 1998, Dr. Sulakvelidze co-founded Intralytix, Inc., in Baltimore—a pioneering U.S. company involved in the development of therapeutic phage preparations for a variety of agricultural, medical, and environmental applications. One particularly strong research area has focused on developing phage preparations and their application strategies for improving the safety of foods contaminated with various foodborne pathogens. Dr. Sulakvelidze has published extensively on the subject and he is the author of one issued and several pending patents related to this field. His phage therapy research has been featured in several magazines and newspapers (including the *Los Angeles Times*, *Newsweek*, *Science*, *Smithsonian,* and *Wired*), and in various radio programs and television documentaries (including National Public Radio's *Science Friday, BBC Radio,* and *Voice of America* radio programs, and a BBC Horizon documentary about phage therapy).

In addition to his work with bacteriophages, Dr. Sulakvelidze is actively involved with the molecular characterization of various bacterial pathogens, particularly *Listeria, Yersinia,* and *Salmonella*. He maintains an active, extramurally funded laboratory at the University of Maryland School of Medicine, where the epidemiology, virulence traits, and genetic composition of those pathogens are being investigated using multilocus sequence typing and other state-of-the-art approaches. Dr. Sulakvelidze serves as an ad hoc reviewer on such journals as *Antimicrobial Agents and Chemotherapy, Applied and Environmental Microbiology, FEMS Immunology and Medical Microbiology,* and the *Journal of Clinical Microbiology,* and for several funding agencies, including the Civilian Research and Development Foundation, International Science and Technology Center, and National Institutes of Health.

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We would like to express our appreciation for their hard work and patience to all the authors who contributed to this book. Particular thanks are due to Hans Ackermann, Karin Carlson, Burton Guttman, and Raul Raya for their thoughtful editorial contributions to other chapters in addition to their own. Also, we gratefully acknowledge the invaluable assistance of our many phage colleagues who provided constructive criticism of various chapters and shared much material prior to publication—particularly to Thomas Häusler, author of *Gesund durch Viren,* and to Richard Calendar and Steve Abedon, coeditors of the 2005 *Bacteriophages,* and the many contributors to their book. We thank Arnold Kreger for his editorial assistance with many chapters. We also are very grateful for the patience and support of our families, colleagues, students, CRC editors, and friends throughout the writing process and to the board of the PhageBiotics Foundation for their support. Special thanks also go to editorial assistant Gautam Dutta at Evergreen for his yeoman's work with the figures, formatting, bibliographies, and details of submission, and for maintaining our Web page (www.evergreen.edu/phage), which will include material to complement the book, such as color versions of many figures and a section for sharing and discussion of methods.

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1 Introduction

Elizabeth Kutter and Alexander Sulakvelidze

Bacteriophages are ubiquitous in our world—in the oceans, soil, deep sea vents, the water we drink, and the food we eat. They are the most abundant living entities on earth—the estimates range from 10^{30} to 10^{32} in total—and play key roles in regulating the microbial balance in every ecosystem where this has been explored. The more information that is generated about the biology, ecology, and diverse nature of phages, the more exciting the field becomes, and the more obvious it is that we still know surprisingly little about them beyond the intricate details of a few model systems. Since their independent discovery by Frederick Twort and Felix d'Herelle in 1915 and 1917, respectively, bacteriophages have been studied in numerous laboratories worldwide, and they have been used in a variety of practical applications. As interest grows in the possibility of using them as antimicrobial agents in a variety of clinical and agricultural settings, they are increasingly capturing the public's as well as scientists' imagination—and we fully expect that a growing number of students and seasoned investigators from various fields will become involved with this exciting field. Thus, our primary purpose in writing this book was to make phage research more accessible for people with a range of backgrounds by providing an overview of the broad depth of phage knowledge and literature, from research techniques and basic molecular biology, to genomics, to applications in agriculture, human therapy, and biotechnology.

In 1959, Interscience Publishers, Inc., published a book entitled *Bacteriophages* that quickly became the most widely used reference source for novice and experienced phage researchers around the world. The book was largely written by Mark Adams; however, when he died suddenly in 1956, many of the best-recognized names of that era in the biological sciences (including Max Delbrück, Alfred Hershey, Gunther Stent, Jim Watson, Tom Anderson, Seymour Benzer, Francois Jacob, Eduard Kellenberger, and George Streisinger) took on its completion—in an effort that strongly emphasizes both the breadth of interest in phage research and the strength and cohesiveness of the phage community at that time. In his preface to *Bacteriophages*, Max Delbrück wrote: "Phage research, after a fitful history during its first twenty years, had all but died out in the middle 1930's. In the textbooks of bacteriology, the bacteriophages, if they were mentioned at all, figured as a curiosity item, unconnected with the rest and disposed of in a couple of pages at most. Today, phage research is vigorously pursued in many outstanding laboratories" (Adams, 1959). Indeed, during the years surrounding the release of *Bacteriophages*, the results of phage research were very

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important in making some of the most significant discoveries in the history of the biological sciences, including the identification of DNA as the genetic material, the discovery of the transduction phenomenon, the deciphering of the genetic code, and the discovery of messenger RNA, as documented in "Phage and the Origins of Molecular Biology" (Cairns et al., 1966).

The intense collaborative application of physical, genetic, biochemical, physiological, and morphological techniques resulted in excellent books about a few model phages (mainly those infecting *E. coli* and *Salmonella*) which dealt with topics ranging from genetic mechanisms and enzymology to structure-function relationships and the details of complex morphogenesis—including whole books about bacteriophages Lambda (Hershey, 1971; Arber et al., 1983), T4 (Mathews et al., 1983; Karam et al., 1994), and Mu (Symonds et al., 1987). In addition, much of the phage community collaborated to produce *The Bacteriophages* (Calendar, 1988), a 1200-page compendium of detailed information about a variety of phages, a new edition of which is in press (Calendar, 2005). Other books explored such subjects as phage-induced enzymes (Cohen, 1968), phage genetics (Birge, 1981; 2000) and phage taxonomy (Ackermann, 1987), or collected key papers on bacterial viruses (Stent, 1965) from the burgeoning literature in the field. During the 1980s, much of the focus of phage research shifted to the development of new technologies exploiting phages and their enzymes; for example, the phage enzymes and vectors that made genetic engineering possible, phage display technologies, and using phages to detect bacterial pathogens—as discussed in Chapters 9 and 11. However, the distribution and functioning of phages in the natural world, their role in general microbial ecology, and their potential therapeutic applications were largely ignored until relatively recently. In the field of phage ecology, as in molecular biology, the publication of a key monograph played a substantial role in stimulating general interest and new research. In that regard, Goyal et al. (1987) provided the first integrated view of the distribution of phages in a variety of environments (e.g., seawater, soil, food, sewage, and wastewater resulting from various industrial processes), and they outlined common methodological approaches used to detect and analyze phages in those environments. Chapters 5, 6, 10, and 13 explore the current rapid developments in this field, which have been greatly facilitated by new genomic and metagenomic approaches as well as by advances in instrumentation and other methods of detection and analysis.

Today, phage research is experiencing a second renaissance because of the new appreciation of phages' ubiquity and prevalence in nature and because of a rekindled public and scientific interest in potential phage applications against antibiotic-resistant bacteria, as well as in various basic and applied aspects of phage biology. Thus, Max Delbrück's introductory statement is quite possibly as applicable today as it was in 1959, when *Bacteriophages* was first published. Indeed, phage research is currently being pursued in many laboratories worldwide, the number of phage therapy- and basic phage biology-related publications is on the rise, and several state-of-the-art technologies are being developed on the "bacteriophage platform." In addition, recent popular books such as *The Killers Within* (Shnayerson and Plotkin, 2002), *Gesund durch Viren: ein Ausweg aus der Antibiotika-krise* (Häusler, 2003), and *Félix d'Herelle and the Origins of Molecular Biology* (Summers, 1999) have explored the history of phage discovery and early phage therapy applications,

as well as the current issues and concerns of antibiotic resistance and potential applications of phages. Public interest has been further increased by documentaries from the BBC and CBC, Dateline Australia, and the Canadian Discover Channel, along with many phage therapy-related articles in the popular press. The American Society for Microbiology has hosted a specialized phage meeting for the first time (entitled "The New Phage Biology") in the summer of 2004—while the Cold Spring Harbor Phage and Microbial Genetics meetings, the Phage and Virus Assembly Meetings, the Evergreen International Phage Biology meetings, and other more specialized gatherings continue to bring together new and old researchers in the field. Furthermore, special sessions dealing with phage applications are being held at many meetings and symposia worldwide, including the 2002 International Virology Conference in Paris, the 2003 International Food Technology Association Meeting, and the Dairy Council's 2003 meeting on agricultural problems of antibiotic resistance and a major German physicians' conference on new approaches to severe wound infections in Dec. 2004. Steve Abedon's Bacteriophage Ecology Group website, www.phage.org, and the Evergreen site, www.evergreen.edu/phage or http://phage.evergreen.edu, provide wide ranges of phage information and links to other sites.

In contrast to the various more specialized phage books, this book integrates the historical phage story with the technicalities of phage research and little-known details about their widespread therapeutic applications in various times and places. Within this general framework, the book also presents basic information about phage biology, phage ecology and genomics, the roles of phages in bacterial evolution, the molecular mechanisms of phage interactions with bacteria and what such studies have taught us about general biological principles, and phage applications in a variety of fields. Thus, our goal has been to focus on integrating the various threads of basic and applied phage research, with the help of colleagues from a range of fields, and to help students and new investigators discover their primary areas of interest and exploration. With this latter objective in mind, we have included an Appendix that details the classical and modern techniques for studying phages—probably the first such compendium since the Adams's book, complementing the more detailed methods described by Carlson and Miller (1994) in *The Molecular Biology of Bacteriophage T4*. Phage research is a field where researchers at all levels can potentially make significant contributions to the body of knowledge. It is relatively easy and inexpensive to isolate and initially characterize phages from a variety of environmental sources; thus, phages are powerful teaching tools for guiding both beginning and advanced students into understanding complex biological systems and their interactions, and they provide many fertile areas for collaborations that may result in exciting and potentially very important practical applications. For example, a joint project between high schools and a leading phage lab that added greatly to our understanding of mycobacterial phages was recently featured on the cover of *Cell* (Pedulla et al., 2003). We hope that this book will help scientists from a variety of backgrounds to better understand the history and excitement of phage research, access the prodigious literature in the field, and explore new applications of phages—the "good viruses" (Radetsky, 1996) that are the most abundant living entities on Earth.