

BACTERIOPHAGES: BIOLOGY AND APPLICATIONS

Elizabeth Kutter and Alexander Sulakvelidze

Table of Contents

- Forward** -- Bruce Alberts
- 1. Introduction**
Elizabeth Kutter and Alexander Sulakvelidze
 - 2. Bacteriophage Research: Early History**
William C. Summers
 - 3. Basic Phage Biology**
Burton Guttman, Raul Raya, and Elizabeth Kutter
-Box 1: Antigenicity of Phages -- Ketevan Gachechiladze
 - 4. Bacteriophage classification**
Hans-W. Ackermann
 - 5. Genomics and Evolution of Tailed Phages**
Harald Brüssow and Elizabeth Kutter
 - 6. Phage Ecology**
Harald Brüssow and Elizabeth Kutter
 - 7. Molecular Mechanisms Of Phage Infection**
Elizabeth Kutter, Raul Raya, and Karin Carlson
 - 8. Bacteriophages and Bacterial Virulence**
E. Fidelma Boyd
 - 9. Phage for the Detection of Pathogenic Bacteria**
Catherine E.D. Rees and Martin J. Loessner
 - 10. Control of bacteriophages in industrial fermentations**
Sylvain Moineau and Céline Lévesque
 - 11. Phage as vectors and targeted delivery vehicles**
Caroline Westwater and David A. Schofield
 - 12. The Use of Phage Lytic Enzymes To Control Bacterial Infections**
Vincent A. Fischetti
 - 13. Phage Therapy in Animals and Agribusiness**
Alexander Sulakvelidze and Paul Barrow
 - 14. Bacteriophage Therapy in Humans**
Alexander Sulakvelidze and Elizabeth Kutter
- Appendix. Working With Bacteriophages: Common Techniques And Methodological Approaches**
Karin Carlson
Box 2: Electron Microscopy – Hans-W. Ackermann

FOREWORD

Bruce Alberts

President, National Academy of Sciences; Washington, DC
University of California, San Francisco; San Francisco, CA

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 that 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 evermore 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 number 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 between 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 can not 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 it 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 21st 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 coauthors 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 their work has established for the many exciting years of research ahead.

Bruce Alberts

President, National Academy of Sciences; Washington, DC 20037

Professor of Biochemistry and Biophysics, University of California, San Francisco; San Francisco, CA 94143

REFERENCES

Alberts, B. M., 2003, DNA replication and recombination. *Nature* **421**: 431-435.

Alberts, B. M. and Fineberg, H. V., 2004 Harnessing new science is vital for biodefense and global health. *Proc Natl Acad Sci U S A* **101**: 11177.

Harrison, S. C., Alberts, B. M., Ehrenfeld, E., Enquist, L., Fineberg, H. V., Mcknight, S. L., Moss, B., et al., 2004 Discovery of antivirals against smallpox. *Proc Natl Acad Sci U S A* **101**: 11178-11192.

Institute of Medicine, 2003. *Microbial Threats to Health: Emergence, Detection, and Response*. Mark S. Smolinski, Margaret A. Hamburg, and Joshua Lederberg, Editors. Natl. Acad. Press, Washington, D. C.

National Research Council, 1996. *National Science Education Standards*. Natl. Acad. Press, Washington, D. C.

Pedulla, M. L., Ford, M. E., Houtz, J. M., Karthikeyan, T., Wadsworth, C., Lewis, J. A., Jacobs-Sera, D., et al., 2003 Origins of highly mosaic mycobacteriophage genomes. *Cell* **113**: 171-182.