

Bacteriophages: 100 years of the history of studies on model viruses in molecular biology

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INTRODUCTION

One hundred years ago, in 1915, Frederick Twort has published an article in which he described factors that could destroy bacterial cells [1]. In fact, he did not know at that time that these factors are viruses, however, retrospective analyses show undoubtedly that he found bacteria-infecting agents, known today as bacteriophages. The history of further studies on these viruses is fascinating, and underlines the role of model organisms in development of molecular biology, as well as the importance of deep understanding of their functions for applications of biological subjects. In this short editorial article, we will present an overview on the history of studies on bacteriophages and their importance in current molecular biology and medicine.

DISCOVERY OF BACTERIOPHAGES

At the beginning of 20th century, the existence of viruses as agents infecting animals and humans has already been known, however, their biology was poorly understood. At that time, Frederick Twort, an

English bacteriologist working at the Brown Institution in London, presented an idea of the existence of non-pathogenic viruses. He assumed that, as in the case of other infectious microbes, there are pathogenic and non-pathogenic variants of viruses. The problem he recognized was that obtaining of the proof of existence of such microbes could be difficult as pathogenicity was the only evidence of the presence of viruses [1]. Therefore, he tested several hundred media, trying to isolate non-pathogenic viruses that would grow under laboratory conditions. Today we know that such attempts could not be successful, and in fact, Twort was not able to culture viruses *in vitro* [1]. Nevertheless, he made some interesting observations. When trying to find non-pathogenic viruses, he used smallpox vaccines in order to detect the “essential substance” which might allow them to grow outside of the host organism. The vaccines used by Twort were contaminated by staphylococci, and he obtained colonies of these bacteria on nutrient plates. Interestingly, he observed that some of these colonies contained glassy areas, suggesting a destruction of their parts. Interestingly, the material from these areas could be passed through porcelain filters and transmitted to other colonies causing again their destruction. Today we interpret these results as discovery of bacteriophages, though Twort assumed that the “bacteriolytic agent” (as he named it), capable of destruction of bacterial colonies, was an enzyme (probably secreted by bacteria) rather than a newly discovered virus infecting bacterial cells [1].

While Frederick Twort did not suspect he discovered bacteriophages, another scientist, Felix d’Herelle, interpreted similar observations as the presence of viruses that infect bacterial cells. He found the clearance of bacterial cultures in the presence of filterable agents which could be transmitted to another culture, causing similar effects [2]. In fact, it was d’Herelle who introduced the name “bacteriophage” [3]. He conducted experiments on bacteriophages for many years, including those leading to introduce the bacteriophage therapy as a specific treatment of infectious diseases caused by bacteria [4]. The idea was quite simple: since bacteriophages destroy bacteria, they could be used as anti-bacterial agents in infected patients. Although d’Herelle’s proposal was

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heavily criticized during the 1930s, and even assumptions have been presented that there were enzymes or toxins rather than viruses which killed bacteria [5], the experiments of “the father of bacteriophages” provided the basis for current attempts to use bacteriophage therapy as an alternative to antibiotics. The apparent gap in studies on bacteriophage therapy (excluding a few countries, in which this kind of treatment was investigated anyway, like former Soviet Union, Poland and France) was caused by discovery of antibiotics, hence, for many years a possible medical use of bacteriophages was forgotten or ignored.

Despite the problems experienced by d’Herelle in introducing bacteriophage therapy, the bacterial viruses became quite quickly recognized as excellent models for biological studies on the molecular level. Their results will be summarized briefly in the next section.

GOLDEN ERA AND DARK TIMES OF STUDIES ON BACTERIOPHAGES

In early 1940s, bacteriophages have been recognized as potentially very useful subjects of studies in the areas of molecular biology and genetics. Max Delbruck, Alfred Hershey and Salvatore Luria formed the “phage group” which attracted biologists interested in investigating bacteriophages as model viruses. In 1945, the first “Phage Meeting” was conducted in Cold Spring Harbor, and subsequent meetings provided a forum for sharing results of experiments on bacteriophages obtained in different laboratories from various countries.

The list of major discoveries made with the use of bacteriophage models is long, and one would need to write a book rather than a short editorial article to describe most of them. Hence, we want to mention just some milestones and breakthroughs, and refer readers to the recently published article [6] and references therein, for more details. The following facts, which appear obvious or paradigmatic today, have been demonstrated in the ‘help’ of bacteriophages: DNA is a genetic material, viruses can encode enzymes, gene expression proceeds through mRNA molecules, genetic code is based on nucleotide triplets, gene expression can be regulated by transcription antitermination, specific genes encode heat shock proteins, there are specific mechanisms of the regulation of DNA replication initiation based on formation and rearrangements of protein-DNA complexes.

Some bacteriophages played special roles in molecular biology as excellent models. This group includes bacteriophages T4 and λ the *Escherichia coli*-infecting viruses that became paradigms in structural and molecular biology studies [7–9]. The genetic control of the choice between two alternative developmental pathways, lytic and lysogenic, of temperate bacteriophages, particularly λ phage, has been established as the first model for a genetic switch, and was used as an example for molecular

regulations occurring in eukaryotic organisms [10, 11]. Moreover, bacteriophages provided an excellent platform for development of genetic engineering and biotechnology. In fact, first cloning vectors were based on bacteriophages, and widely-used genetic recombination technologies and phage display systems could not be developed without the use of these viruses [12].

Nevertheless, after the “golden era” of bacteriophages, which occurred from mid 1950s to mid 1980s [9], there were dark times for studies on them. When scientists learned about basic molecular mechanisms and basic structures of bacteriophages, many opinions appeared at the end of 20th century that we knew enough about these viruses, that no more breakthroughs could be expected in this field, that bacteriophages have little role in medicine and environment, and that there was a time to stop financing research on them. It was supposed that the switch of the research to more complicated biological systems would be more beneficial than continuation of studies on bacteriophages. After relatively short period of 20 years or so, such opinions had to be verified negatively, which will be described shortly in the following section.

RETURN OF BACTERIOPHAGES AS IMPORTANT SUBJECTS OF BIOLOGICAL, MEDICAL, ENVIRONMENTAL AND BIOTECHNOLOGICAL STUDIES

Last years of 20th century and the beginning of 21st century provided unexpected discoveries which indicated how incomplete our knowledge on bacteriophages was. They also demonstrated and underlined the importance of these viruses in the environment and human health.

Although it was known that many pathogenic bacteria produce toxins, the discovery that some of them are encoded in genomes of bacteriophages, not those of bacteria, was a real surprise. Various pathogenic bacteria are lysogenic, bearing prophages that carry genes responsible for production of toxins. This group of bacteria includes, but is not restricted to, *Vibrio cholerae* (causing cholera) [13], *Corynebacterium diphtheriae* (causing diphtheria) [14], and enterohemorrhagic strains of *Escherichia coli* (causing bloody diarrhea and further severe complications, like hemolytic uremic syndrome) [15]. Therefore, bacteriophages may cause serious medical problems, as toxins encoded in their genomes are dangerous for humans. Moreover, such toxins are usually produced efficiently upon prophage induction, hence, even if bacteria can be killed after treatment with antibiotics, the toxins may be produced efficiently in the meantime. Interestingly, it was proposed recently that systems of prophages with genes coding for toxins could evolve as specific defense modules against unicellular eukaryotic predators rather than as bacterial pathogenicity enhancers [16–18].

Another medical field of bacteriophages' come back is bacteriophage therapy. The method was introduced by Felix d'Herelle [4], then ignored in most of Western countries and almost forgotten, and now it reappears as a chance for treatment of infectious diseases in the era of multiple antibiotic resistance of bacteria. There are still many unsolved problems with bacteriophage therapy, mostly related to procedures, thus, no such treatment is registered now, and it can be used only as experimental therapy [19]. Nevertheless, there are some spectacular successes in the use of bacteriophages for treatment of infectious disorders which provide a hope for further development of this therapeutic strategy [20].

As mentioned above, bacteriophages played a crucial role for development of genetic engineering and biotechnology. Still, novel systems for genetic manipulations [21], detection of target molecules or pathogens [22–24], production of biomedical compounds [25, 26] or displaying peptides of various properties [27, 28] are being created by employing bacteriophage-based tools. Undoubtedly, we can expect development of further sophisticated biotechnological systems based on bacteriophages.

Finally, it is necessary to mention the environmental meaning of bacteriophages. Until recently, we were unaware of the importance of these viruses in ecosystems. However, it is now estimated that there are about 10^{31} bacteriophage particles and about 10^{30} bacterial cells on Earth. Therefore, bacteriophages and their hosts are the most abundant and genetically diverse groups of organisms [29]. As a consequence, viruses infecting bacterial cells play a crucial role in the regulation of any bacteria-dependent environmental processes. One should also remember about specific ecological niches, like mammalian gut. Since the number of bacteria in the human gut exceeds the number of cells forming the human body, bacteriophages infecting intestinal bacteria may significantly influence human physiology and health [30].

CONCLUSION

In this short editorial paper, we have presented briefly milestones of the history of research on bacteriophages, from their discovery, through the golden era and dark times, to the current stage. The first century of studies on these viruses was very fruitful and full of impressive discoveries, as well as sophisticated applications. Despite a short crisis in such studies, now we have recognized that bacteriophages play particularly important roles in natural biological systems, as well as in human health. We suppose that next important discoveries in the area of molecular biology of bacteriophages will appear in near future, which should improve our understanding of biological mechanisms, and stimulate development of biotechnology and medicine.

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The corresponding author is the guarantor of submission.

Conflict of Interest

Authors declare no conflict of interest.

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