AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Bacteriology

EDITORIAL

Classic Spotlight: Phage Bring Punch to the Party

Victor J. DiRita

Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, Michigan, USA

The once-dreaded disease diphtheria, caused by *Corynebacterium diphtheriae*, holds a prominent place in medical, scientific, and social history. The first Nobel Prize in Physiology or Medicine was awarded in 1901 to Emil von Behring for demonstrating the protective effect of antiserum directed against the diphtheria toxin. In the 1920s, the Great Race of Mercy used dogsled teams to bring diphtheria antitoxin over 650 miles of frozen Alaskan terrain to Nome—this event introduced America to a canine hero, one of the lead sled dogs named Balto, and gave us the annual Iditarod dog sled race.

Two *Journal of Bacteriology* papers on *C. diphtheriae* (1, 2), published in the early 1950s by Victor J. Freeman, had a major influence on how we understand bacterial pathogens and the evolution of pathogenicity traits. Freeman, working at the University of Washington School of Medicine, demonstrated conclusively that avirulent nontoxigenic strains of *C. diphtheriae* become virulent and toxigenic after exposure to a bacteriophage. This was the first demonstration of "lysogenic conversion," now well known as the mechanism responsible for the acquisition of important traits in microbes such as *Vibrio cholerae*, *Staphylococcus aureus*, enterohemorrhagic *Escherichia coli*, and many others.

The work arose through Freeman's interest in phage typing *C. diphtheriae* strains. He had in hand two uncharacterized phages from Australia (phage A) and Canada (phage B). Examining five avirulent isolates, he observed strong lytic activity on four of them using phage B and weaker lytic activity on the same four with phage A; the fifth strain was resistant to lysis by either phage (1). When lysates from the phage infections were examined in guinea pigs, Freeman observed necrotic activity in an intradermal assay and lethality in a subcutaneous injection assay. Animals administered anti-diphtheria toxin sera by intraperitoneal injection were protected from subsequent subcutaneous administration of the lysates. Toxin-neutralizing activity of the antiserum in the *in vivo* assays was confirmed by *in vitro* tests showing a strong positive reaction of the sera with phage B lysates. Necropsy findings from animals that succumbed to the injection of the lysates were similar to those previously reported "as characteristic of diphtheria intoxication" (1). In contrast to the lethal effects of the lysates, injection of a purified phage filtrate into the guinea pigs resulted in a locally limited and nonlethal necrosis that resolved without any further effects on the animals.

Stable virulent *C. diphtheriae* strains recovered from phage B infection of the originally avirulent strains were all resistant to lysis by that phage; such immunity is now well understood as being due to production of a repressor by the prophage. However, the strains could be induced to undergo lytic growth, and this yielded a phage that, according to Freeman, "in its gross characteristics, could not be distinguished from phage B" (1). From his studies, Freeman drew the larger conclusion that "apparently, exposure of the phage-susceptible avirulent cultures to phage B resulted in the production of virulent lysogenic strains of *C. diphtheriae*" (1).

Freeman originally speculated that the mechanism of conversion to stably toxigenic strains upon phage treatment was due to spontaneous mutation to toxigenicity and concomitant phage resistance, allowing subsequent elimination of sensitive nontoxigenic siblings by phage lysis. With his colleague I. Una Morse, Freeman went on to publish another paper on lysogenic conversion. That work, also published in the *Journal of Bacteriology* (2), used a remarkable micromanipulator method to study the progeny of single cells. In that paper, Freeman and Morse concluded that "the change to toxin production might well be interpreted as being due directly to the acquired lysogenicity. Conceivably, the bacteriophage may make possible the toxin production through some as yet undetermined association with the metabolic processes of the bacterial cell." Today we understand clearly that Freeman's latter hypothesis was much closer to the mark and that in fact the phage carries the toxin gene.

These incredibly insightful observations published in the *Journal of Bacteriology*—aided by some serendipity (as Freeman himself acknowledged in a later set of reflections on his career [3])—formed the foundation for understanding the impact that bacteriophages have had on the evolution of bacterial pathogens. This impact has become much more widely understood in the ensuing years, and phages and their hosts have become excellent model systems for the study of key evolutionary principles and for the discovery of remarkable new and important biology concepts such as restriction/modification systems and the CRISPR-Cas mechanism of bacterial immunity.

REFERENCES

1. **Freeman VJ.** 1951. Studies on the virulence of bacteriophage-infected strains of *Corynebacterium diphtheriae*. J Bacteriol **61:**675–688.
2. **Freeman VJ, Morse IU.** 1952. Further observations on the change to virulence of bacteriophage-infected avirulent strains of *Corynebacterium diphtheriae*. J Bacteriol **63:**407–414.
3. **Freeman VJ.** 1988. Reflections. National Library of Medicine, Bethesda, MD. http://profiles.nlm.nih.gov/ps/retrieve/ResourceMetadata/BBBCHQ.

Citation DiRita VJ. 2016. Classic spotlight: phage bring punch to the party. J Bacteriol 198:202. doi:10.1128/JB.00644-15.

Address correspondence to diritavi@msu.edu.