Chapter 1
Overview of Bacteriophage Lifecycles and Applications

1 Introduction

Bacteriophages (phages) are well-established bacteria-specific viruses whose discovery is credited to the independent and nearly simultaneous works of Twort (1915) and d'Hérelle (1917) (Summers 1999) in the early 20th century. Each of the researchers characterized phages as the pathogens of bacteria following the hint of much phage-like phenomena from the 19th and 20th centuries. The late 1930s and early 1940s represented the most significant era for phage research and its impact on biological research (Abedon and Thomas-Abedon 2010), including the research by the "Phage Group". This group included the work of Max Delbrück and other highly notable geneticists, including James Watson and Francis Crick (Abedon 2012a). The group quickly established that phage could be used for the treatment of bacterial infections, since called "phage therapy", and were so named. "Bacteriophage" translates to "bacteria eaters".

While phage biology and the study of phage genetics were of interest, it was phage therapy that and its antibacterial potential that was the primary driver for phage research (Hanlon 2007; Summers 2001). Phage therapy however, failed to match the anticipation of its initially envisioned potential, particularly in a time when the phage themselves were poorly characterized, and the approach was thwarted in favour of small molecules in the Western World in the 1950s (Kropinski 2006; Summers 2001).

Although phage-based therapeutics did not meet the expectations of their initial interest, they have played a crucial role in the study of genetics and molecular biology (Henry and Debarbieux 2012), including contributions to the understanding of organisms much more complex than the phage themselves (Campbell 2003; Goodridge 2010). As such, the study of phage may have actually set the stage for its own demise in medicine.

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There has been a strong resurfacing of interest in phage beyond the field of phage therapy and particularly, in phage-based technologies (Citorik et al. 2014; Henry and Debarbieux 2012). Phages offer great potential and impact in the food and agriculture, biotechnology, global nutrient cycling, and human health and disease industries. Furthermore, advances in genetics, bacteriology and synthetic biology have opened many opportunities to further phage-based therapeutics. This chapter provides an overview of phage biology and genetics as the governing principles necessary for the consideration of phage toward phage-based medical applications discussed in this book.

2 Phage Infection and Life-Cycle

Phage infection begins when the virion attaches to its host cell (adsorption) as part of the multi-step process of infection. This is shortly followed by the translocation of phage DNA into the host cell and subsequent expression of the phage genome within the host (Abedon 2012b; Samson et al. 2013). The aftermath of phage infection will depend on the phage, the host, and the circumstances of infection.

Successful adsorption will result in one of four circumstances: (i) the phage lives and replicates to form progeny and the host dies via lytic infection; (ii) both the phage and the host bacterium live and propagate, as seen with lysogeny, imparted by the lysogenic cycle of temperate bacteriophages; (iii) The phage dies and/or does not produce progeny and the host lives, a typical result of infection of hosts encoding restriction endonuclease (Labrie et al. 2010) and/or CRISPR/cas systems (Jiang and Doudna 2015); and (iv) both the phage and the host die as a result of abortive infection system(s) (Olszowska-Zaremba et al. 2012).

2.1 Lytic Phage

Most phage undergo lytic phage infection cycles whereby daughter progeny are produced and released at the expense of host cell lysis and death. These are considered “productive infections” where infections quickly lead to the release of viral progeny. Once the viral genome enters the host cell, phage-encoded genes are expressed in the bacterial cytoplasm, the functions of which, take over host bacterial metabolism (Young 2014). Infecting phages then enter a latent period during which phage particles are assembled and, once a threshold number of virions are produced, phage gene products “holin” and “lysin” (for classical double-stranded DNA phages) are responsible for the destruction of the host cell wall and subsequent release of the phage progeny to the extracellular matrix and neighboring cells (Abedon 2012a; Olszowska-Zaremba et al. 2012; Young 2014).

2.2 Temperate Phage

Bacteriophages that possess the ability to be stably harbored within their host as a prophage, thereby lysogenizing the host, are referred to as temperate. Temperate phages have the ability to switch between the lytic and lysogenic cycles, often existing as a prophage integrated into the host chromosome, but possessing the capacity to enter the lytic cycle in response to host and/or other external danger signals (typically the host SOS response) (Mardanov and Ravin 2007; Roberts and Devoret 1983).

Lysogenic cycles are characterized by two features: (i) the prophage is replicated sufficiently to permit daughter host cells to inherit at least one copy of the phage's DNA; and (ii) infections are not productive in that no structural virions are produced, but rather replication occurs vertically *in tandem* with host replication and division. While integration is perhaps a more common route of lysogeny, a prophage can manifest extra-chromosomally as a stable low copy plasmid that is not integrated into the host genome. Integration requires an integrase, which binds homologous segments of phage and bacterial DNA, resulting in site-specific recombination (Abedon 2012a).

The switch from a lysogenic cycle to productive infection, or lytic cycle, is known as induction or derepression. Prior to induction the phage will only produce proteins needed to maintain lysogeny, normally a repressor(s), necessary to prevent expression of all genes involved in the virus's vegetative growth, but also capable to trigger induction upon receiving the appropriate host/environmental signal(s) (Mardanov and Ravin 2007; Roberts and Devoret 1983).

3 Phage Infection Stages

3.1 Phage Entry

Bacteriophage infection begins when the particle adsorbs to a specific surface or appendage site(s) on a bacterial host cell. This initial recognition process is highly specific and typically involves the specific interaction of a binding ligand as some structural component of the phage with a corresponding receptor(s) on the host cell. Phages of gram-negative bacteria may recognize the polysaccharide moieties (e.g. phage T4) and/or outer membrane proteins (e.g. phage λ, T4) (Gaidelyte et al. 2006; Morita et al. 2002; Randall-Hazelbauer and Schwartz 1973). Gram-positive phages normally attach to the cell surface polysaccharides of the host (Valyasevi et al. 1990) and once a phage adsorbs, phage DNA translocates into the host cytoplasm where phage gene expression and replication may then occur.

3.2 DNA Replication

Viral gene expression ensues once the phage genome has entered its host cell (Abedon and Thomas-Abedon 2010). The phage genes will typically encode the capacity to harvest the host and exploit its metabolism to express its own genes. The specifics of phage DNA replication and the expression of the DNA products depend on the phage infecting the cell and the conditions surrounding the infection, including the species and attributes of the host cell (Mcnerney et al. 2004). The phage genome will typically code for assembly products for the production of phage progeny and for the amplification of its own genome.

3.3 Phage Assembly

Bacteriophages have served as model systems of viral assembly for the last half-century. Similar to phage DNA replication, the formation of phage particles and their individual structures will differ with each phage strain. However, despite extensive genetic diversity between phage genomes, similarities remain in structure and viral life cycle between bacterial viruses. These viral particles are essentially made up of two components: nucleic acid and a protein shell or capsid. Formation of the particles requires specific protein-protein and protein-nucleic acid interactions in addition to a well-established set of conformational changes resulting from each of these interactions, all of which are specific to the phage strain type (Aksyuk and Rossmann 2011). Examples from each of the major classes of phage (by nucleic acid genome), dsDNA, ssDNA, and ssRNA phage are described below in terms of phage assembly:

3.3.1 Tailed (dsDNA) Phages

All tailed bacteriophages' host ranges are determined by the specialized tail organelle. Despite a number of distinct strategies, most phages contain cell binding receptor proteins that bind host cells and trigger DNA release from the head. Phages with tails are from the *Caudoviridae* family and can be characterized by tail morphology as either short tails (*Podoviridae*), long non-contractile tails (*Siphoviridae*) or long contractile tails (*Myoviridae*) (Abedon 2012a; Ackermann 2003). Tailed phages follow several distinct steps for phage assembly: (i) assembly of a prohead, or the shell of capsid protein with a portal allowing for (ii) the packaging of DNA using ATP energy, (iii) maturation of proheads and (iv) attachment of the neck and tail proteins or a preassembled tail (Ackermann 2007).

3.3.2 ssDNA Phages

Filamentous phages from the *Inoviridae* family, including M13, fd and f1, are male (F plasmid)-specific ssDNA phages and represent some of the simplest viral entities on earth. Filamentous phages assemble into rods from five different structural proteins, the length of which is proportional to the size of the genome. Generally, the proteinaceous helical rod consists of approximately 2700 copies of the major capsid protein with pentamers of pIII and pVI on one end and pVII and pIX on the other (Abedon 2012a; Ackermann 2003).

3.3.3 ssRNA Phages

Single-stranded RNA phages from the *Leviviridae* family include phages MS2, f2 and ΦCb5. In this morphological group, the highly specific *Leviviridae* capsid is characterized by ninety dimers of the capsid protein arranged into an icosahedral lattice. The virions also assemble with a maturation protein on the capsid to mediate phage attachment and the phage genome is packaged inside the capsid upon maturation (Aksyuk and Rossmann 2011).

While phages have been classified according to morphology it is important to note that inter- and intra-genic modules of information can be combined to perpetually generate new "species" of phage due to co-infection and recombination. As such, phages that share structural similarity may be comprised of vastly different genetic systems for propagation and sustainability.

4 Hurdles for Phage-Based Therapeutics

The small-size, genetic malleability and ease of production of bacteriophages make them ideal candidates for many biotechnological applications. However, no therapeutic has ever been produced without a limitation(s) and phage are no exception. Perhaps one of the major obstacles facing the use of phages for clinical applications is the perception of viruses to the public as "enemies of life" thus imparting a lack of enthusiasm towards phage-based therapeutics (Merabishvili et al. 2009). This issue is further complicated by the documented previous failures in phage-based therapeutics, where phages were used unsuccessfully as antimicrobial agents—an outcome related much more to the lack of understanding of the phage themselves rather than the potential of the technology.

Two critical points in the use of phage-based therapeutics are necessary to address in order to make a substantial impact in the field: (1) improving passaging capacity to create long-circulating phage that can evade the mammalian immune systems; and (2) generating efficiencies in phage scale-up and manufacturing processes.

Phages are quickly removed from a mammalian host by the reticuloendothelial system (RES), a part of the innate mammalian immune system (Lu and Koeris 2011).

New drug delivery technologies, including polymer-based coatings have been shown to enhance phage uptake and reduce phage inactivation/clearing by the RES (Goodridge 2010; Lu and Koeris 2011). In vitro/in vivo evolution of phages could also be used to produce nanoparticles with enhanced properties, including decreased clearance by the host immune system (Merril et al. 1996; *see Chap. 7 for further discussion on phage immune responses and immunomodulation*).

Issues with phage immunity are further complicated by the phage manufacturing and isolation processes. While phage manufacturing has reached a sophistication level worthy of clinical grade products (Merabishvili et al. 2009; Strój et al. 1999; Tanji et al. 2004), isolating phages from their bacterial hosts is convoluted by the presence of endotoxins and pyrogens that are released during phage-induced lysis (Lu and Koeris 2011). As such, there is currently a dearth of well defined and safe manufacturing protocols (Merabishvili et al. 2009) to form safe and stable formulations (Lu and Koeris 2011). Merabishvili et al. (2009) were the first to successfully demonstrate a small-scale, laboratory-based production and application of bacteriophage cocktails system to overcome some of the prevailing issues associated with the efficiency of phage isolation and purification—most notably, the use of a commercially available endotoxin removal kit able to attain efficient purity needed for a European clinical trial (Merabishvili et al. 2009). This group, among others, have addressed these issues (Górski et al. 2005; Yacoby and Benhar 2008), though standard manufacturing procedures are still in demand and are required before phage-based therapeutics can be marketable as legitimate biologics.

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