Temperate Phages, Prophages, and Lysogeny

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Abstract

Prophages form with their host a very special type of interaction called lysogeny. There they are able to help the host, but they are also able to kill their hosts, producing their own virion progeny during the process. The phage-host interaction itself is very complicated with many different mechanisms, some of which served as important bases for our understanding of molecular biology. Because of these mechanisms, some prophages are extensively studied and now are considered to be model organisms. Other prophages have caught our attention because they have managed to turn their hosts into deadly pathogens by delivering payloads of toxin genes along with genes encoding other bacterial virulence factors. Many prophages are also able to cross species borders, facilitate horizontal gene transfer, and otherwise give rise to the creation of bacteria, via lysogenization, with new capacities not necessarily observed before. Due to improvements in sequencing technologies, we are now discovering how widespread and important the interaction of prophages with their hosts is in nature. In this chapter some aspects of their biology, interactions with hosts, and contribution to pathogenesis is described.

Introduction

Not all phage infections, even if successful, progress directly to virion maturation and release. Instead, it is possible for certain phages, which are called temperate, to infect bacteria, persist, and replicate, not explicitly as viruses but instead as a capsid-free genetic element. In this state the phage genome instead is described as a prophage and the phage-infected bacterium a lysogen. Prophages and associated lysogens can stably persist over long time spans, but as infections by a virus, nevertheless retain a potential to transition to the production of virion progeny via a process known as prophage induction. Thus, free phages can give rise either to what are known as lysogenic cycles or instead to productive cycles (the latter, e.g., lytic cycles), with the transition between these different states variously described as lysis-lysogeny, lytic-lysogeny, or lysis-lysogenic decisions. While productive cycles give rise to free phages, lysogenic cycles give rise either to continued lysogenic cycles or instead, following induction, to productive cycles. While in the lysogenic state, prophages can contribute to the phenotype of the hosting bacterium, displaying such phenomena as superinfection immunity and lysogenic conversion.

This chapter summarizes some basic knowledge about lysogeny and prophages, including the mechanisms they use to assure their survival, properly interact with their hosts, and otherwise augment host capabilities. Previous reviews on this subject include, e.g., those of Łoś et al. (2010), Casjens and Hendrix (2015), and Howard-Varona et al. (2017). In this volume see also chapter ▶ "Phage Infection and Lysis"

as well as chapter ▶ "Bacteriophage-Mediated Horizontal Gene Transfer: Transduction". Here we consider the basic, general aspects of lysogeny and prophages as well as aspects associated with specific temperate phages.

Lysogeny and Lysogenic Cycles

Lysogeny is a long-lasting partnership between a bacterial cell and an infecting phage. The phage at least temporarily gives up its ability to produce virions, while the bacterium generally tolerates and even may benefit from the phage's presence. Such bacteria in association with these phages are called lysogens, and phages which are able to display such lysogenic cycles are referred to as temperate. The vast majority of temperate phages are also lytic phages, in terms of their productive cycles, though chronically releasing temperate phages are known as well. Most infections by temperate phages seem to result in virion-productive infections; lysogenic cycles nevertheless will also begin with the adsorption of a temperate phage virion to a susceptible bacterial cell, which upon entrance into lysogeny takes on a form, in terms of its genome, known as a prophage.

Stably Associating with Host Bacteria

Temperate phages display a variety of ways to stably maintain their prophage in host bacterial cells. This can involve direct integration of the genetic material of prophage with bacterial chromosome, but also prophages exist in the form of stable, extrachromosomal elements, such as plasmids (Fig. 1). Stable maintenance of prophages in the form of a plasmid is more demanding than chromosomal prophages as every cell division may result in losing of the plasmid by one of the daughter cells. That tendency, however, can be counteracted by existing as multicopy plasmids, but this in turn will create an increased burden for host cells, which may cause the lysogen to lose, e.g., a competitive edge against other bacteria. Some temperate phages have evolved very sophisticated sets of mechanisms to force host cells to stably maintain a prophage copy. One of these prophages, which lysogenize cells in the form of a plasmid, is phage P1, which uses at least two mechanisms to ensure stable prophage maintenance. These mechanisms are described in detail in a separate chapter of this book. Briefly, they consist of a partitioning system, which is able to deliver a single copy of prophage to each daughter cell and a toxin-antitoxin addiction system, which is capable of killing daughter cells which have failed to acquire a copy of the prophage plasmid (Yarmolinsky 2004).

Some temperate phages bypass the problems with plasmid inheritance simply by evolving a way to integrate into the bacterial chromosome, and thus "outsourcing" to the host the whole work of prophage genome replication and segregation to daughter cells. The process of integration of the prophage genome into the chromosome is usually carried out by highly specialized integrases, which will insert the prophage genome into the predefined integration sites, as occurs in the case of phage lambda (Hendrix 2002), but the process may be much more random and conducted by

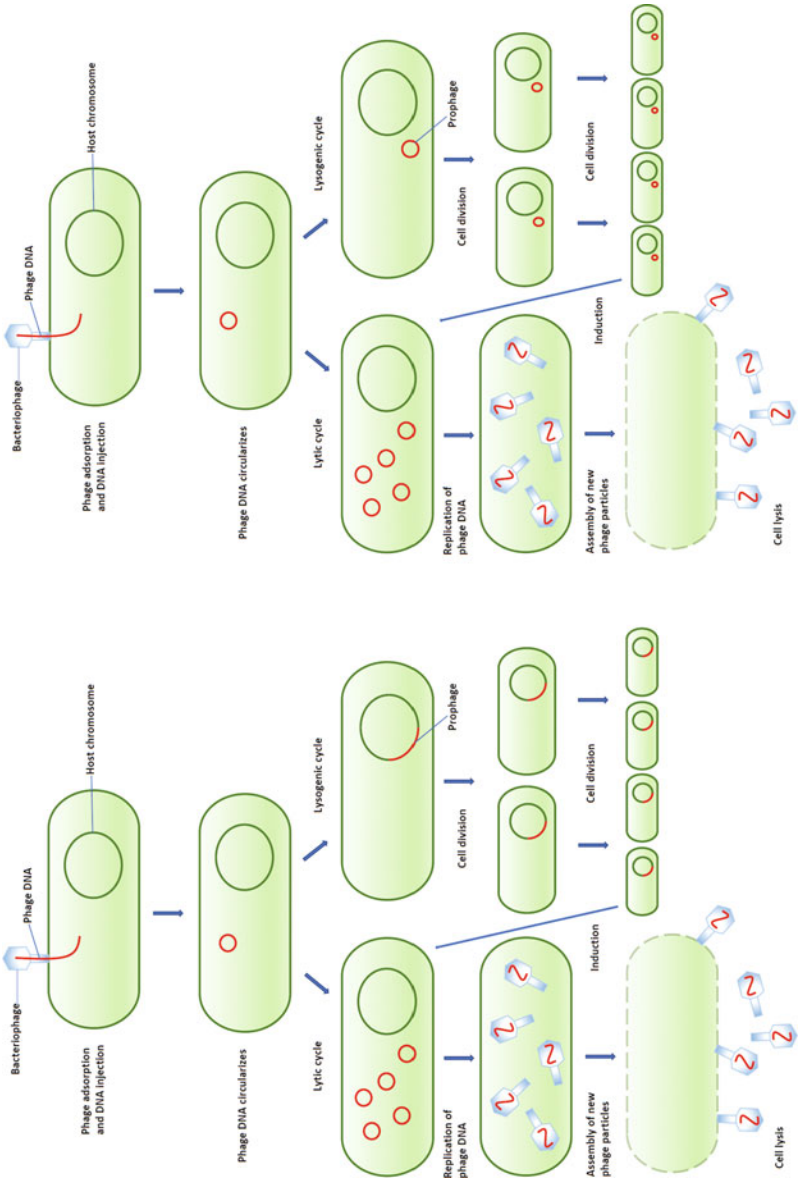

Fig. 1 Developmental pathways of temperate phage integrating into chromosome (left panel) and integrating with a cell as a plasmid (right panel)

transposases as in a case of phage Mu (Ranquet et al. 2005). Many temperate phages integrate into bacterial chromosomes sometimes in multiple locations. Some of them, in a multi-chromosome bacterial species, may integrate to a few chromosomes at the same time. An example of this is CTX^{cla}, a cholera toxin encoding prophage which is integrated into both chromosomes of *Vibrio cholerae* in classical biotype strains (Kim et al. 2014, 2017).

Regardless of the form of the prophage within the host cell, there is a general scheme of maintaining lysogeny, which may be considered to form the basis of a temporary partnership. Following infection of the host cell, prophages need to silence a vast majority of their genes, due in part to the majority of them not being needed in the prophage state (e.g., structural capsid genes) but also that some of them may encode products toxic to the host (e.g., prophage lambda *kil* gene). The silencing of those genes is maintained by the phage repressor or repressors, which are able to suppress activity of promoters needed for release of phages from lysogeny, which is then followed by progress toward further development steps. Very often, repressors are sophisticated molecular sensors capable of recognizing molecular signals, which then release prophages from repression in response to such signals. In the case of many prophages, mostly represented by large groups of lambdoid phages, such a signal is a triggering of SOS response regulons, which cause repressors to self-destruct in the process of autoproteolysis (McCabe et al. 2005). For other prophages, such as those of phage Mu or P2-like prophages, even though the prophage-inducing signal is not known and not synchronized, massive induction has been observed in case of these phages (Ranquet et al. 2005). Release of prophage repression leads to activation of productive cycle-associated promoters and thus progression of the development cycle toward production of progeny virions and destruction of the host cell.

Benefiting the Lysogen

The interaction of the prophage with a host cell may be beneficial for both sides, as prophages usually have lysogenic conversion genes and morons onboard. These genes, while not contributing to the development cycle of prophage, nevertheless are active during the whole lysogeny period and provide some beneficial functions for the host cells, allowing them to perform better in certain situations or environment. Moreover, repressors of prophages may also modulate host cell metabolism, directing it into more effective and competitive use of the available resources, often resulting in faster growth of the lysogenized host cells, when compared to their prophage-free counterparts (Chen et al. 2005). These types of interactions will be discussed more in following paragraphs. Regardless of mutual benefits, lysogeny can have deadly output for each side, as both of them – prophage and bacteria – are trying to outsmart each other. Prophage is constructed in such a way to eventually be induced in response to problems which may appear in the cell. As so, it is a kind of molecular time bomb, which while offering temporary benefits, host bacteria will eventually be triggered. Bacterial cells, however, have mechanisms of reducing

unnecessary genetic content, which will, in a random way, in a process of natural selection, try to get rid of or at least disable the dangerous part of prophage while keeping the beneficial part at its own disposal. The driving DNA deletion mechanism is random, but those bacterial cells, which managed to disable prophage and thus turn it into cryptic prophage, while keeping its beneficial elements, may obtain an advantage over their counterparts, which do not contain prophage, or in which the prophage contained is fully functional and thereby able to eventually kill the host cell. The resulting cryptic prophages may be then identified in bacterial chromosomes like, e.g., in *E. coli* K12 (Campbell 1998).

A prophage is a phage genome which has come to occupy the interior of a bacterial cell, all the while retaining the potential to produce new, progeny virions. But so long as induction has not taken place, they do not actually produce those new virions, indeed any virions. Prophages thus are an example of a bacteriophage lifestyle which is not a direct part of a productive cycle. The genetic association of prophages with their hosts at the first glance, however, would seem to be a parasitic interaction, as prophages utilize host resources in order to multiply their genomes. However, it is in the interest of the prophage – sitting inside of these cells – to not so-cripple its host, which could have the effect of allowing other bacteria to outcompete that lysogen. Rather, prophages can fine-tune and equip their hosts with numerous useful tools, i.e., in terms of lysogenic conversion as introduced above. In the case of bacteriophage lambda, but also many other prophages, the metabolism of the cell is modulated by inhibition of the gluconeogenesis pathway (Dykhuizen et al. 1978; Chen et al. 2005), which allow lysogenized bacteria to grow faster and more efficiently and to outcompete their prophage-free counterparts.

Lysogenic cycles at first glance may look like they are not very productive for the phage, but in fact, prophages are reproduced and transferred to every daughter cell, and thus, in the long run, can be more productive than simple lytic cycles. Prophages during multiplication of host cells nevertheless may be induced in a small fraction of a lysogen population, but even this small frequency of induction allows phages to keep their presence as virions in environments, sometimes even at a relatively high level. In fact, this strategy may ensure very effective spread in the environment (Rotman et al. 2010) which is due to the potentially much higher number of progeny phage particles. It can be easily explained by the fact that phage, by killing a single cell, may produce up to ~1000 progeny phages, but the same cell lysogenized by phage may produce billions of daughter cells, all of them carrying prophage. Let us say that among them, thousands of cells will undergo spontaneous inductions, releasing phage progeny, which gives an effective productivity from a single infection event at the level of millions of phage particles.

Lysis-Lysogeny Decisions

Entering into a lysogenic cycle by temperate bacteriophage is a serious commitment. This decision, made soon after a temperate phage's adsorption to a host bacterium, binds the fate of the phage with that bacterium, so has to be made carefully, and

chosen particularly when benefits of lysogeny seem to be higher than the benefits of immediate use of host resources to build progeny phage particles. Lysis-lysogeny decisions, perhaps not surprisingly, therefore can also be molecularly very complex. The process of choosing the developmental pathway by phage has been best studied in bacteriophage lambda, where it is still not fully understood, and thereby additional pieces of the puzzles continue to be added to the already complex picture. In the case of this phage, the decision is made on the basis of several signals derived from levels of cAMP and ppGpp alarmones, which indicate the physiological state of the cell. In general, the lower if the level of cell resources and/or the poorer the environment, then the more probable will be the choice of prophage state (Słominska et al. 1999). The rationale behind this is that the more resource-starved the cell, then the fewer progeny it can immediately produce, and thus it may be wise to wait for better times via lysogenic cycles, meanwhile improving host cell performance by fine-tuning host metabolism and adding additional functions by the process of lysogenic conversion, which may help the host, e.g., to colonize a new ecological niche.

Another signal, which can be sensed by incoming temperate prophage, is the density of bacteriophages in the environment. In the case of bacteriophage lambda, the more phages which infect the cell in short period of time, then the more likely will be the choice of lysogenization instead of lytic development by the phage. This may be explained by the fact that multiple infections by phage during the lysis versus lysogeny decision may indicate very high local phage densities, which in turn suggest that the local environment is already saturated with phage and thus number of uninfected host cells may be very low. In such situations the most rational choice would be to lysogenize the cell and to reserve cell and its progeny resources for potential production of the progeny phages (Avlund et al. 2009). Recently it was discovered that some phages use an active communication system showing how many cells were lysogenized in the environment. It is achieved by the secretion of very short signal peptides, with concentration influencing the decision of lysis versus lysogeny. High enough levels of these peptides in particular promote lysogeny (Erez et al. 2017). Various methods of communication between phages outside the cell and phages already occupying the cell were summarized by Abedon (2017).

Induction

Once taking on the form of a prophage, the virus in addition to expressing lysogenic conversion genes and otherwise replicating in tandem with its host also waits for the molecular signal to be induced. Prophages, that is, can be considered to be potentially only temporary elements of a host cell. All prophages, in other words, sooner or later have up to four possible fates: ongoing existence as a functional prophage (though its sequence will not necessarily remain constant, i.e., prophages in principle can evolve while remaining genetically intact), death in the course of death of its host, inactivation as functional prophage (thus become cryptic prophages, but also outright curing, i.e., prophage loss in full) despite ongoing host survival, or induction. Inactivation may be a result of deletions or mutations within prophage genomes caused by the activity of host enzymes or simple error in DNA handling. Induction,

in turn, is prophage ability to enter a productive development cycle and thereby use host resources to produce progeny phages.

As noted above, prophages, to prevent development toward a lytic pathway, need to silence genes responsible for this lytic development. This suppression is achieved by repressor proteins, which are constitutively expressed during lysogeny. They not only suppress the activity of lytic cycle-associated promoters but can regulate activity of their own promoter, in order to keep repressor-protein concentrations within certain ranges. Very often repressors are sophisticated molecular sensors capable of recognizing molecular signals and releasing prophages from repression in response to such signals.

Immunity

As noted, prophages after lysogenization of the host cell produce repressor proteins, which keep them in a prophage state and prevent activation of lytic promoters. Each lysogenized cell must contain enough repressor to prevent each of their prophages from being induced. Otherwise the cell will host a lytic cycle resulting in lysis and release of new virion progeny. A cell once lysogenized is immune to infection with the same phage, which is called homoimmunity, or any phage containing the same immunity region, which state is called heteroimmunity. An immunity region is a complete set of elements containing repressor gene and operators, which are sequences to which repressor proteins bind. Operators are responsible for proper placing of repressors within or in proximity of the promoter region, and thus they are responsible for silencing or enhancing that promoter activity. If the DNA of a phage using the same repressor is injected into a cell, then their operators will immediately be occupied with repressors typical for that prophage (Yarmolinsky 2004; Ptashne 2004). This will block any further development of that phage toward a lytic cycle, and in majority of cases it will also block proper prophage establishment (lysogenic cycle), as transcription of some early genes responsible for, e.g., prophage integration, will also be suppressed. Phage genome injected into lysogenized host with the same immunity may still be able to form a lysogen, however, but in many cases with very low frequency (Fogg et al. 2010).

Lysogenic Conversion and Morons

Lysogenization of bacteria with a prophage very often causes the host bacterium to gain new properties that are beyond those associated simply with lysogenization. Most frequently the modifications are due to expression of lysogenic conversion genes which allow bacteria to gain new properties and potentially colonize new ecological niches. Prophages, directly by lysogenic conversion and indirectly by participating in the spread of genomic islands, including pathogenicity islands,

consequently can greatly contribute to the diversification of bacterial strains, including in terms of the emergence of highly virulent pathogens.

Lysogenic conversion can be defined as a temperate phage-associated heritable change in the host cell's genotype and phenotype that is not caused by simply packing host DNA instead of phage genome in the process of generalized transduction and is independent of the effects expected from repression and integration and other phenomena related to the lysogenic state. The latter, not lysogenic conversion-related changes, can include immunity to superinfection or loss of bacterial functions caused by the insertion of phage into host genes (Łoś et al. 2010). Lysogenic conversion can be, but strictly need not have been, caused by morons, which are defined as an additional gene in a prophage genome, often acquired by horizontal gene transfer, without direct function in mediating either a phage's lysogenic or lytic cycle (Brüssow et al. 2004). The function of some morons is elusive and may not even be easily observed as a lysogenic conversion. On the other hand, some lysogenic conversion effects, e.g., blocking of the gluconeogenesis pathways by phage repressors, are not caused by morons. This is a cause, when genes, which are essential elements of bacteriophage development or prophage maintenance, are responsible for additional effects on host recognized as a lysogenic conversion. Thus, even though there is an overlap in lysogenic conversion and the function of morons, these two terms have to be treated as a separate phenomenon (Table 1).

Lysogenic conversion was discovered in 1951 with the observation that diphtheria toxin is encoded on the prophage genome integrated into *Corynebacterium diphtheriae* (Freeman 1951). Since then, bacteriophage-encoded toxins have been found in a range of both Gram-positive and Gram-negative strains, including *Escherichia coli*, *Shigella* spp., *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Clostridium tetani*, *Clostridium botulinum, Staphylococcus aureus*, and *Streptococcus pyogenes* (Barksdale and Arden 1974; O'Brien et al. 1984; Huang et al. 1987; Nakayama et al. 1999; Betley and Mekalanos 1985; Weeks and Ferretti 1984; Goshorn and Schlievert 1989). The focus of researchers on pathogens caused a sort of bias, which may suggest that lysogenic conversion is a phenomenon mostly observed in pathogens. It is very likely, however, that the truth is the opposite, with pathogens best studied in comparison to environmental bacteria. Moreover, lysogenic conversion which leads to change in the pathogenicity of host strain is relatively easy to observe. That is not a case in environmental strains, which we often cannot propagate in laboratory or draw too many conclusions from by observation in their natural environments, versus the comparatively straightforward and easy to monitor environments consisting of pathogens causing disease.

In this chapter different aspects of prophage biology were discussed. It is important to stress, that all above mentioned features of prophages are never observed as a complete feature set of single prophages. Instead, each one is utilizing a few of them. In subsequent paragraphs different examples of prophages and their biology will be discussed, to show how these mechanisms make a complete and fully functional molecular program allowing prophage to interact properly with its host.

Table 1 Differences and similarities of lysogenic conversion and the action of morons

	Lysogenic conversion	Action of morons
Causative agent	Additional or native gene or genetic element in phage	Additional gene in phage
Integral part of the phage cycle?	No	No
Phenotypic change?	Yes	Yes or no
Causative element disposable for phage?	Yes or no. Ability to dispose element may be conditional	Yes

Bacteriophage Lambda

Bacteriophage lambda was discovered over 60 years ago and became one of the most important model organisms in molecular biology. Basic molecular mechanisms of crucial cellular processes and regulation of development were investigated using bacteriophage lambda as a model. Many similar phages called lambdoid bacteriophages have since been isolated and characterized. These phages have a similar genome organization to that of phage lambda, and they can recombine with lambda to make biologically functional hybrids (Casjens and Hendrix 2015). Grose and Casjens (2014) defined the lambda supercluster as a group of temperate *Enterobacteriaceae* phages whose encoded functions are syntenic with the phage lambda genome and whose transcription pattern and gene expression cascade are similar to that of lambda. In this supercluster are only *Enterobacteriaceae* phages. Others which are similar to lambda phage but infect different bacteria have some of their genes, in many cases lysis genes, which are not syntenic with those of the lambda supercluster (Grose and Casjens 2014). There are also phages which differ substantially in nucleotide sequences from lambda but have a lambda-like lifestyle with similar transcription regulatory mechanisms (Hendrix 2002).

The Lysogeny Decision

One of the most studied features of lambda phage is the molecular switch, which is responsible for the lysis versus lysogeny decision. Despite the fact, that the mechanism of this switch is generally quite well understood, there are still many unknowns in the fine-tuning of the decision-making driven by it. In general, the process starts after the phage genome has entered the bacterial cell, when the bacteriophage makes a decision whether to produce new progeny phages and lyse the host cell, or instead to form a prophage and lysogenize the cell. Lytic development pathway is a default for the phage. Switching to the lysogeny pathway during phage lambda development depends on a level of accumulation of the phage lambda CII protein, since this protein is a transcriptional activator that stimulates strong *cI* gene transcription from the lambda phage pE promoter. CI repressor, the product of *cI* gene, is required to maintain lysogeny state. It

forms multimers that bind simultaneously to three operators known as OL and three others known as OR to repress the early promoters. Moreover, synthesis of Int protein that catalyzes insertion of the phage lambda dsDNA genome into the host chromosome is stimulated by CII transcriptional activation of the phage lambda promoter, pI (Węgrzyn and Węgrzyn 2005). Expression of phage lysis genes is also inhibited by activity of the cII-dependent paQ promoter which in turn directs the production of antisense mRNA for the Q protein, necessary for late genes expression (Hoopes and McClure 1985).

Quantity of CII is also influenced by cell physiology, environmental factors and number of phage particles infecting the cell. Lysogenization is much more frequent in a starved bacterium. The CII protein is very unstable and degraded by the host-encoded FtsH protease (Shotland et al. 2000). During starvation, production of cyclic AMP is started. This results in inhibition of FtsH activity and increased stability of CII. Also, another nucleotide alarmone – ppGpp – regulates the amount of FtsH protease and modulates cII-mediated activation of pE and pI promoters (Słominska et al. 1999). Additionally, ppGpp negatively regulates pR promoter activity that results in inhibition of the lytic development of phage lambda (Potrykus et al. 2002). In slowly growing bacteria, polyadenylation of mRNA by poly (A) polymerase increases, resulting in lower stability of mRNAs. Expression of cII gene is negatively regulated by *oop* RNA transcript that after polyadenylation is degraded, and the cII gene therefore is more effectively expressed (Szalewska-Palasz et al. 1998).

Another factor controlling lysis-lysogeny decisions is temperature. Formation of CII, which is active in tetrameric form, depends on temperature. At lower temperatures the process of multimerization is more effective. Also, FtsH-dependent degradation of CII tetramers is less effective than monomers (Shotland et al. 2000). Stability of CII protein depends on phage-encoded CIII protein that is also a substrate for FtsH protease (Herman et al. 1997). Expression of the cIII gene is under control of pL promoter that is more active at lower temperatures (Giladi et al. 1995). Moreover, the transcript for cIII forms two alternative structures and only one of them is able to bind to the 30S ribosomal subunit and initial translation. The proportion of these two structures depends on temperature, and at lower temperatures there is more transcripts that are able to bind to the ribosome (Altuvia et al. 1989). Lysogeny is also favored during high multiplicity of infection. When a low number of phages infect the cell, injection of phage DNA takes place on cellular poles. Interestingly, it was found that the majority of FtsH that degrades CII is located in these regions. On the other hand, during high multiplicity of infection, injection of phage DNA takes place on whole cell surface (Edgar et al. 2008).

Lambda and Lambdoid Genomes

Phage lambda genome is 48,503 bp long, and upon lysogenization it is inserted into bacterial chromosome between the *Escherichia coli gal* and *bio* genes. The integration is reversed during the induction process, and the prophage genome is excised. Usually this process is very precise, but sometimes the prophage is not excised

properly and chunks of host DNA flanking attachment sites may be taken away, replicated together with phage DNA and packed into produced phage capsids, thus allowing for specialized transduction of flanking DNA (Rolfe 1970) (chapter ▶ "Bacteriophage-Mediated Horizontal Gene Transfer: Transduction"). Lambda and lambdoid prophages carry powerful recombination systems, which make it much easier for them to exchange whole, functional gene blocks, and as a result these phages possess genomes which are highly mosaic. Although occasionally their sequence may not be very similar to each other, they share general similarity in genome structure (gene synteny), even though they may belong to different phage families, as lambda belongs to family *Siphoviridae*, while a majority of Shiga toxin containing lambdoid phages seem to belong to phage family *Podoviridae* (Smith et al. 2012). Lambdoid prophages very often deliver to the host various morons (i.e., additional DNA fragments originating originally from outside of phage genome) and lysogenic conversion genes (chapter ▶ "Bacteriophage-Mediated Horizontal Gene Transfer: Transduction"), which can dramatically change the lysogenized host properties. The grim reputation of a fraction of lambdoid bacteriophages is caused by their ability to carry Shiga toxin genes, which can turn otherwise quite innocent *E. coli* strains into deadly pathogens (Smith et al. 2012). Phage lambda, which is a commonly used model in scientific laboratories and seems to be harmless, nevertheless encodes a serum resistance gene, *bor*, which in may also augment the *E. coli* host, potentially contributing to its formation into a pathogen (Barondess and Beckwith 1995).

Prophages P2 and P2-Likes

Phage P2 is a temperate bacteriophage isolated by Prof. Giuseppe Bertani in 1951 from the Lisbonne and Carère strain of *Escherichia coli*, together with phage P1 and P3. (Ackermann 1999). Since then a large number of P2-like phages have been isolated, but P2 is still the most well-described member of the family, along with phage 186. The term P2-like phages is used for phages that share some but not all features with P2 phage. Some of the most known members of P2-like phages are the noted 186 along with phages HP1, HK239, and WΦ. Classification of the P2-like family is based on characteristics like serological relatedness, host range, lack of inducibility by ultraviolet irradiation, inability to recombine with phage lambda, a unique class of cohesive DNA ends on chromosomes, and also the possibility to support growth of satellite phage P4.

Genome sequencing has shown that P2-like phages are relatively common in nature and can multiply in different γ-proteobacteria. P2 phage itself can infect most strains of *E. coli*, as well as *Serratia marcescens*, *Salmonella typhimurium*, *Klebsiella pneumoniae*, or *Yersinia* sp. It was shown that in *E. coli* reference library, ECOR (Ochman and Selander 1984), about 30% of the strains encloses P2-like prophages (Nilsson et al. 2004). Upon infection p-phage P2 can derepress phage P4 lysogens via the action of the P2 Cox protein (below), which acts as transcriptional activator of the late P4 promoter, Pll, which is required for P4 replication.

P2 Virion and Genome

The P2 virion consists of an icosahedral head 60 nm in diameter and complex tail of 135 nm with a contractile sheath. During the coinfection with phage P4, it produces a smaller 45 nm head (Dokland et al. 1992). The baseplate at the end of the tail contains six tail fibers and a spike to cling to the bacterium during the adsorption. Free phage P2 adsorbs during the infection to the core region of the lipopolysaccharide of *E. coli* and injects its DNA into the cytoplasm.

The entire genome has been sequenced (Gene Bank accession number AF063097) and the double-stranded DNA molecule consists of 33.6 Kb, with 19-nt-long cohesive ends (Linderoth et al. 1991). The cohesive ends allow for circularization of genome after infection (Bertani and Six 1988), one of them is fastened to the tail, at the head-tail attachment site, which prevents circularization inside the capsid (Chattoraj and Inman 1974). The 42 phage P2 genes can be organized into 3 essential classes. One class contains genes involved in lysogenization, another class of genes necessary for DNA replication, and a third class consisting of genes encoding structural proteins and lysis functions. Moreover, P2 includes a number of open reading frames (ORFs) that may encode functional proteins.

P2 Lysogeny

After the start of infection, phage P2 can enter into two different life cycles, either lytic growth or lysogeny. The choice of life cycle depends on the promoter that takes control. The early promoter, Pe, controls lytic growth and the Pc promoter controls the genes involved in lysogenization. The balance between the phage-encoded repressor proteins, C and Cox, is responsible for determining the outcome of the lysis versus lysogeny decision. This control is termed a transcriptional or development switch. The promoter Pc is repressed by Cox (meaning ***C***ontrol ***o***f e***x***cision), which is the first gene controlled by the promoter, Pe, which prevents the expression of the genes necessary for lysogeny (Saha et al. 1987). During this repression, the phage can enter into its lytic cycle and start the transcription of early genes. When repression of the Pe promoter by C protein occurs, the P2 genome instead is inserted into the host chromosome by site-specific recombination. Since the C repressor is not inactivated by the SOS/RecA system of *E. coli*, the prophage cannot be induced by ultraviolet irradiation.

The mechanism of integration of P2-like phages is quite similar to λ site-specific recombination. P2-like phages are able to integrate into host chromosome with the use of a phage integrase protein, the histone-like protein, IHF, and both phage attP and bacterial attB attachment sites (Frumerie et al. 2005). P2 phage often integrates at a specific site. Recombination occurs between 27-bp-long sequence (core) and host chromosome.

The transcriptional switch of temperate phages must be set in order to control lysogenization and lytic cycle after infection. In phage P2, promoters Pc and Pe are

facing each other. The Pc promoter directs transcription of the C repressor which is responsible for downregulation of the Pe promoter, active during lytic growth. On the contrary, the Pe promoter, which commands the lytic growth, directs transcription of the Cox protein resulting repression on the Pc promoter. The Pc transcript also encodes the integrase and the Pe promoter controls the expression of proteins needed for DNA replication. The decision between lytic and lysogenic life cycle appears to be the consequence of the relative concentration of the Cox protein and C repressor. C and Cox repressors are able to downregulate opposite promoters at relatively low concentrations, but also their own promoters, when proteins are present at high concentration, which prevents unnecessary buildup of these proteins (Saha et al. 1987).

The common characteristics of P2-like phages is that two face-to-face-oriented promoters control the proper functions of cycles by directing the transcriptions of two transcripts, which partially overlap without any overlap in genes regions. Although the transcriptional switches of the P2-like phages have similar arrangements, they still can vary within the group. Phage HP1 has two early promoters, P_{R1} and P_{R2} (Esposito et al. 1997), and depending on promoter used the transcript overlap can be respectively 44 bp or 72 bp (Esposito et al. 1997). In phage 186 the overlap is about 60 bp and P2 transcripts have the shortest overlap of about 35 bp. Phage 186 has an additional gene, cII, that controls establishment of lysogeny but not its maintenance. This gene is located in early operon, encoding activator that acts on promoter P_E (Lamont et al. 1993; Neufing et al. 2001). The phage Wϕ also contains two face-to-face promoters, but the repressors of Wϕ bind to two directly repeated operators, which differ in comparison to P2 operators (Liu and Haggård-Ljungquist 1999). Cox proteins of phages P2, P2 Hy *dis*, and WΦ have been shown to be multifunctional, since they not only act as repressors of Pc. They can also perform as directionality factors for site-specific recombination that inhibit integration and promote excision of phage genomes in or out of the host chromosome (Nilsson and Haggård-Ljungquist 2007).

Temperate Phages of Staphylococci and Streptococci

Staphylococcal Phages

Morphological Families and Classification

The majority of described staphylococcal phages infect *Staphylococcus aureus* and were first used for the typing of clinical isolates. High interest in finding and characterizing *S. aureus* phages was caused by the fact that this common human pathogen is responsible for many nosocomial and community-acquired infections. Also, a growing number of antibiotic-resistant strains contributed to extensive studies of *S. aureus* phages. In this species, temperate bacteriophages appear to be widespread as every strain of *S. aureus* sequenced so far contained at least one prophage. Several studies report isolation of phages from coagulase-negative

species, like *Staphylococcus epidermidis*, *Staphylococcus hominis*, or *Staphylococcus saprophyticus*. However, only small number of these phages have been sequenced, characterized, and studied. It is probably due to the fact that pathogenesis of coagulase-negative *Staphylococci* relies on factors required for their commensal mode of life instead of toxins that are often encoded by prophage genes, where instead the latter is for *S. aureus*.

A majority of temperate staphylococcal phages belong to the *Siphoviridae* family in the *Caudovirales* order. They are composed of icosahedral capsid (morphotype B1, like coliphage λ) or prolate capsid (morphotype B2) and noncontractile tail with a baseplate structure with double-stranded DNA as a genetic material (Brüssow et al. 2004). Early classification of staphylococcal phages, both strictly lytic and temperate, was based on their lytic properties, morphology, and on genome size and organization. Based on classification proposed by Brüssow and Desiere, staphylococcal phages belonging to *Siphoviridae* were classified as Sfi21-like or Sfi11-like phages by some authors (Brüssow and Desiere 2001). Recently, the classification of staphylococcal Siphoviridae was updated based on phylogenetic relationships (Gutiérrez et al. 2014; Adriaenssens et al. 2018, 2020). Most of the total of 200 bacteriophages with completed genome sequences available in public databases were assigned to six genera. Classification of the remaining phages is an ongoing process.

The Triavirus genus includes nearly 30 phages that infect *S. aureus*. All viruses belonged to morphotype B2. Proteomic analysis indicated presence of group-specific proteins like A-type polymerase or unique capsid and tail proteins. RinA protein is also present instead of RinB homolog that is present in other phages (Gutiérrez et al. 2014). RinA and its homolog are proteins responsible for phage-mediated packaging and transfer of virulence genes (Ferrer et al. 2011). Genus Biseptimavirus includes at least three *S. aureus* phages with a similar genome size and morphology corresponding with B1 morphotype. These phages share characteristics with the other genera, such as the presence of nucleases, similar to those from the "Triavirus" genus, and a common morphotype with "Phietavirus." Phietavirus is a genus that includes at least 36 staphylococcal phages. Those phages show B1 morphotype (with an exception of EW phage), and they also show similar sequence of genes in tail morphogenesis module. Among peptidoglycan hydrolase domains, a CHAP domain and a glucosaminidase domain are present, instead of a lytic transglycosylase SLT domain and a peptidase_M23 domain, that were detected in other two genera (Gutiérrez et al. 2014). Six phages, referred by authors as "orphan phages," remained unclassified as they lack clear homology to members of three proposed genera (Gutiérrez et al. 2014). However, the present method of classification is not recognized by ICTV (International Committee on Taxonomy of Viruses).

Two other genera of staphylococcal Siphoviridae: Fibralongavirus, Sextaecvirus include 4 phages each. One staphylococcal siphovirus, namely SP-beta, which infects Staphylococcus epidermidis was asignet to Spbetavirus genus together with Bacillus phage Z.

Streptococcal Phages

Morphological Families and Classification

Most streptococcal bacteriophages known, as of this writing, were isolated from β-hemolytic *Streptococci* group A. It is speculated that around 90% of these streptococci may contain temperate phages (Hynes et al. 1995). Phages infecting *Streptococcus pneumoniae* were also described (Romero et al. 2009), but there are only a few known prophages of other *Streptococcus* species. Some have been found to carry virulence factors like antibiotic resistance or toxins, while others appear to have no effect on the phenotypes of their hosts. Furthermore, despite various studies, still little is known of molecular biology of streptococcal phages. Phages induced from lactic streptococci were described to have isomeric heads and noncontractile tails (Huggins and Sandine 1977), therefore matching *Siphoviridae* B1 morphotype. Phages isolated from various strains of *Streptococcus pneumoniae* showed the same morphotype. Experiments confirmed that genetic material of some known staphylococcal phages is ds-DNA (Romero et al. 2009).

Most of the *Streptococcus* phages have not been classified yet. Based on classification methods proposed by Brüssow and Desiere, and on phylogenetic relationships there are three defined genera that include streptococcal phages: Brussowvirus (phages 2972, 858, ALQ132, O1205, Sfi11), Moineauvirus (phage DT1, DT1.1, DT1.2, DT1.3, DT1.4, DT1.5, phiAbc2, Sfi19 and Sfi21), and Saphexavirus (phage SPQS1) (Brüssow and Desiere 2001; Adriaenssens et al. 2018, 2020). The latter includes several Enterococcus phages in addition to a single Streptococcus phage).

Role of Prophages in Modulation of Bacterial Host Biology and Evolution

Formerly, bacteriophages have been mostly perceived as parasites of bacterial cells as well as convenient tools to investigate the genetics of bacteria. However, the newest data of complete bacterial genome sequences has revealed the crucial role of prophages in the diversity of strains among bacterial species. Nowadays we can observed three main trends which have renewed the interest in phage research: (1) phage influence the cycling of organic matter in the oceans (chapter ▶ "Bacteriophage Ecology"), (2) they are potential tools for the treatment of antibiotic-resistant bacterial pathogens (section "Therapeutic Use of Bacteriophages"), and (3) they have major impact on bacterial short-term evolution (chapter ▶ "Bacteriophage-Mediated Horizontal Gene Transfer: Transduction") (Canchaya et al. 2003).

Previously discussed lysogenic conversion is only one of at least five different ways by which temperate phages affect bacterial fitness; however, they also can (1) serve as anchor points for host genome rearrangements, (2) disrupt host genes, (3) protect lysogens from lytic infection, (4) lyse competing strains following prophage induction, and (5) can introduce new fitness factors (lysogenic conversion, transduction) – (Table 2) (Brüssow et al. 2004). Mechanism of gene propagation, including virulence factor genes such as those encoding toxins, adhesins or

aggressins, is critical for the emergence of new pathogenic strains. Recent studies suggest that a large amount of genetic information in natural environment and in bacterial genomes is of phage origin (Muniesa et al. 2011). On the other hand, phage integration leading to loss of function can be observed, e.g., in case of *Staphylococcus aureus*, in which L54a and φ13 prophages integrate into the chromosome and cause the inactivation of a lipase and a β toxin gene, respectively (Fortier and Sekulovic 2013), or phage Mu integration, which occurs in random places by transposition and may lead to inactivation of genes and modification of their expression (Harshey 2014).

Some bacteria have evolved to exploit the presence of prophages for their own purposes. This usually occurs through crippling the prophage by deletion and then evolving prophage remains by the host for the new function. In *Pseudomonas aeruginosa*, two phage-tailed gene clusters have developed into bacteriocins (Nakayama et al. 2000). The defective *Bacillus subtilis* prophage PBSX has maintained the capacity to build a size-reduced phage head into which 13 kb fragments of random bacterial DNA are packaged turning it into gene transfer agent (Canchaya et al. 2003). Such use of defective prophages seems to be quite common in nature, as reviewed by Redfield and Soucy (2018). Perception of especially temperate bacteriophages thus has changed. They are now seen more as a "versatile carrier of genetic information within and between bacterial species and as a means of rearranging existing genetic information into unique combinations. Comparative bacterial genomics has revealed the 'mutualistic' role of bacteriophages in the evolution of bacterial pathogens" (Boyd and Brüssow 2002)

Toxins and Virulence Factors Encoded by Prophages

Arguably the most studied aspect of prophages influencing host biology was their impact on formation of pathogens. Below, a few examples of this influence are discussed.

Staphylococcus aureus

Most of virulence factors present in *S. aureus* were delivered by phages. Toxins and antibiotic resistance have been identified in other *Staphylococcus* species, although their role in pathogenesis remains unknown. In general, individual phages carry only a single virulence factor gene, although there are exceptions. *S. aureus* phage phiSa3 and its relatives, for example, may encode up to five virulence factors (Goerke et al. 2006). Virulence factor genes appear not to be strictly associated with one specific phage and most probably are exchanged by horizontal gene transfer and recombination. In most phages, virulent genes are located near phage attachment site (*attP*) and downstream the lysis module (Fig. 2). It is consequently speculated that they were obtained by phages by aberrant excision events from a bacterial chromosome (Wagner and Waldor 2002) (chapter ▶ "Bacteriophage-Mediated Horizontal Gene Transfer: Transduction").

Table 2 Bacterial virulence factors dependent on phage (on the basis of Brüssow et al. 2004)

Bacterial host	Protein	Gene	Phage
C. botulinum	Neurotoxin	C1	Phage C1
C. diphtheriae	Phage C1	tox	β-Phage
E. coli	Shiga toxins	stx1, stx2	H-19B
	Enterohaemolysin $FC3208	hly2	φFC3208
	Cytolethal distending toxin	cdt	Unnamed
	OMP[b]	bor	λ
	OMP	eib	λ-like
E. coli O157	Superoxide dismutase	sodC	Sp4, 10
M. arthritidis	Vir	vir	MAV1
N. meningitidis	Membrane proteins	Mu-like	Pnm1
P. aeruginosa	Cytotoxin	ctx	φCTX
P. multocida	Mitogenic factor	toxA	Unnamed
S. aureus	Enterotoxin	see, sel	NA
	Enterotoxin P	sep	φN315
	Enterotoxin A	entA	φ13
	Enterotoxin A	sea	φMu50A
	Exfoliative toxin	eta	φETA
	Leukocidin	pvl	fPVL
	Leukocidin	pvl	φPVL
	Staphylokinase	sak	φ13
S. canis	Mitogenic factor	Unnamed	phisc 1
S. enterica	Glucosylation	rfb	ε34
	Glucosylation	gtr	P22
	Type III effector	sopE	SopEΦ
	Type III effector	sseI (gtgB)	GIFSY-2
	Type III effector	sspH1	GIFSY-3
	Superoxide dismutase	sodC-I	GIFSY-2
	Superoxide dismutase	sodC-III	Fels-1
	Neuraminidase	nanH	Fels-1
	Virulence factor	gtgE	GIFSY-2
	Antivirulence factor	grvA	GIFSY-2
S. flexneri	O-antigen acetylase	oac	Sf6
	Glucosyl transferase	gtrII	SfII, SfV, SfX
S. mitis	Phage coat proteins	pblA, pblB	SM1
S. pyogenes	Toxin type A	speA	T12
	Toxin type C	speC	CS112

(continued)

Table 2 (continued)

Bacterial host	Protein	Gene	Phage
	Superantigens	speA1, speA3, speC, speI, speH, speM, speL, speK, ssa	8232.1
	Hyaluronidase	hylP	H4489A
	Phospholipase	sla	315.4
	DNase/ streptodornase	sdn, sda	315.6, 8232.5
	Mitogenic factors	mf2, mf3, mf4	370.1, 370.3, 315.3
V. cholerae	Cholera toxin	ctxAB	CTX$

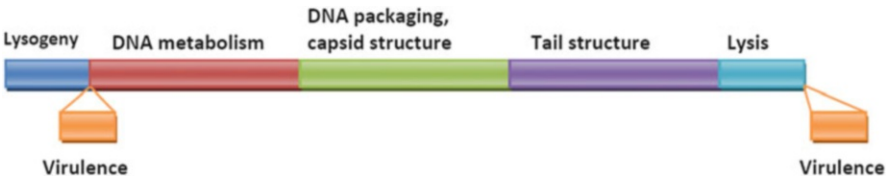

Fig. 2 Organization of staphylococcal phages of *Siphoviridae* family as presented by Deghorian and Van Melderen (2012). Five functional modules arranged as follows: lysogeny (dark blue box), DNA metabolism (red box), DNA packaging and genes encoding capsid proteins (green box), genes encoding tail proteins (violet box), and cell host lysis genes (light blue box). If present, genes encoding virulence factors are localized downstream lysis genes or inserted between lysogeny and DNA metabolism genes

S. aureus is a leading cause of gastroenteritis, resulting from the absorption of staphylococcal enterotoxins after consumption of contaminated food (Le Loir et al. 2003; Łoś et al. 2010). *S. aureus* is also the leading cause of mammary gland infections in dairy animals. Specific antibiotic-resistant strains cause epidemics in hospital settings (Brüssow et al. 2004). Many skin infections such as furunculosis, staphylococcal scalded skin syndrome, and wound infections are caused by this bacterium. *S. aureus* can cause a wide range of diseases, ranging from toxicosis, such as food poisoning, to invasive diseases. *Staphylococcus aureus* strains encode a large variety of secreted toxins, and these toxins (Table 3) are responsible for most of the clinical symptoms associated with the infections. Among known *S. aureus* virulence factors carried by phages are staphylokinase, Panton-Valentine leukocidin (PVL), enterotoxin A, and exfoliative toxin A. Staphylokinase is a 136-aa-long protein carried by phage ϕ13. Its binding with host-produced plasminogen results in the formation of active plasmin, a proteolytic enzyme facilitating bacterial penetration into the surrounding tissues (Bokarewa et al. 2006). Panton-Valentine leukocidin (PVL) is a cytotoxin present in the majority of *Methicillin-resistant Staphylococcus aureus* (MRSA) and encoded in a prophage designated as ϕ-PVL. It produces two toxins, known as LukS-PV and LukF-PV that act together as subunits. PVL causes

leukocyte destruction and necrotic lesions of skin and mucosa. It is responsible for severe and often lethal necrotizing pneumonia (Lina et al. 1999).

The staphylococcal exfoliative toxins (ETs) are extracellular proteins that cause splitting of human skin at the epidermal layer during infection in infants and causes blistering skin disease and staphylococcal scalded skin syndrome (SSSS) (Ladhani et al. 1999). These diseases affect mainly infants and children, with severity varying from localized blisters filled with fluid to general exfoliation that can affect entire body surface. Two antigenically distinct toxins possessing identical activity have been isolated from *Staphylococcus aureus*, ETA and ETB, which are serologically distinct (Kondo et al. 1975). The gene for ETA (eta) is located on the chromosome, whereas that for ETB is located on a large plasmid. Relatively few clinical isolates produce ETA which during early research suggested that *eta* gene is acquired by horizontal gene transfer. Indeed, exfoliative toxin A (ETA) gene is carried by ϕETA phage. ETA toxin is 242 aa long, has a molecular mass of 26,950 Da, and is heat-stable. It consists of two domains, S1 and S2, each consisting of six-strand β-barrels and a C-terminal α-helix. It has serine protease-like properties, binds to the skin protein profilaggrin, and cleaves substrates after acidic residues (Yamaguchi et al. 2000). Temperate phage (ϕETA) that encodes ETA was isolated. ϕETA has a head with a hexagonal outline and a noncontractile and flexible tail. The genome of ΦETA is a circularly permuted linear double-stranded DNA, and the genome size is 43,081 bp. ΦETA converted ETA nonproducing strains into ETA producers. Southern blot analysis of chromosomal DNA from clinical isolates suggested that ΦETA or related phages are responsible for the acquisition of genes in *S. aureus*.

Staphylococcal bacteriophages are also responsible for mobilization of *Staphylococcus aureus* pathogenicity islands (SaPIs). They are chromosomal DNA segments acquired by horizontal transfer that rely on a helper phage for moving (Tallent et al. 2007). SaPIs are known to carry various virulence factors such as gene encoding toxic shock syndrome toxin or variants of von Willebrand factor-binding protein that provides *S. aureu*s with ability to coagulate host blood plasma (Lindsay et al. 1998). SaPIs can be replicated and mobilized as a response to SOS-induced excision of a helper prophage, by the infection of host cell by phage or by the joint entry of SaPI and a phage. SaPIs may require certain phage in order to be mobilized, although some phages as phage 80α are known to mobilize all known SaPIs (Łoś et al. 2010).

Lysogenic conversion of staphylococci associated with expression of virulence factors was first reported in the early 1960s (Blair and Carr 1961; Winkler et al. 1965). Over 40 years ago discovered some *S. aureus* toxin: a phage could convert nontoxigenic strains to alpha-hemolysin production. The staphylococcal enterotoxin A gene, *sea*, was mapped near the attachment site of the temperate phage PS42-D (Betley and Mekalanos 1985; Brüssow et al. 2004). Southern hybridizations revealed that the *sea* genes in staphylococcal strains were associated with a family of phages rather than with one particular phage.

Phage PVL encodes a bicomponent cytotoxin, the Panton-Valentine leukocidin. The two toxin genes *lukS* and *lukF* were located between the phage lysin gene and the right attachment site (Kaneko et al. 1998). The two toxins assemble into pore-forming transmembrane complexes and lyse their target cells, human

Table 3 *Staphylococcus aureus* virulence factors dependent on phage (on the basis of Helbin et al. 2012)

Toxin/pathogenicity determinant (gene)	MGE	Description/mechanism/ symptoms
Protein responsible for biofilm formation (*bap*)	SaPIbov	Biofilm formation inside of cattle udder, involved in *mastitis* symptoms
Von Willebrand factor-binding protein (vWBps) host-specific variant	SaPIbov2, SaPIbov4, SaPIbov5, SaPIeq1, SaPIov2	A variant of genomic vWBp protein, cattle, or equine plasma coagulation
CHIPS, Chemotaxis Inhibiting Protein of *S. aureus* (*chip*)	φ13, φtp310–3, φ252B, φMu3A, φN315, φNM3, φSa3JH1, φSa3mw, φSa3 ms, φSa3JH9, φSa3USA300, φβC-USA300_TCH1516	Decreases activity of C5AR1 and FPR1 neutrofile receptors relative to C5a complement system component and formylated peptides
Exfoliative toxin A (*eta*)	ΦETA	toxin responsible for staphylococcal scalded skin syndrome symptoms
Enterotoxin A (*sea*)	ΦSa3ms, ΦSa3mw, Φ252B, ΦNM3, ΦMu50a	*Staphylococcal enteritis*
Enterotoxin B (*seb*)	SaPI1, SaPI3	*Staphylococcal enteritis*
Enterotoxin C (*sec*)	SaPIn1, SaPIm1, SaPImw2, SaPIbov1	*Staphylococcal enteritis*
Enterotoxin K (*selk*)	ΦSa3ms, ΦSa3mw, SaPI1, SaPI3, SaPIbov1, SaPI5	*Staphylococcal enteritis*
Enterotoxin L (*sell*)	SaPIn1, SaPIm1, SaPImw2, SaPIbov1	*Staphylococcal enteritis*
Enterotoxin P (*selp*)	ΦN315, ΦMu3A	*Staphylococcal enteritis*
Enterotoxin Q (*selq*)	ΦSa3ms, ΦSa3mw, SaPI1, SaPI3, SaPI5	*Staphylococcal enteritis*
PVL, Panton-Valentine Leukocidin, bisubunit toxin (*lukS* and *lukF*)	ΦSA2pvl, ΦSLT, ΦPVL, ΦSA2MW, ΦSA2usa	Causes the lysis of mammalian leukocytes, stimulates overproduction of proinflammatory factors leading to tissue necrosis
SCIN, Staphylococcal complement inhibitor (*scn*)	φ13, φN315, φ252B, φNM3, φMu50A, φSa3JH1, φSa3 ms, φSa3mw, φSa3JH9, φMu3A, φtp310–3, φSa3USA300, φβC-USA300_TCH1516	Intervenes in bacterial cells opsonization through the inhibition of complement system C3bBb convertase
Staphylokinase (*sak*)	phl3, ph42D, phφC, φN315, φMu50A	Disrupts the bacterial cells phagocytosis, a component of plasmin which helps the Staphylococcus cells to penetrate and proteolytically decay the tissues
Toxic shock syndrome Toxin-1, TSST-1 (*tstH*)	SaPI1, SaPI2, SaPIbov1, SaPI3	Main toxin responsible for TSS stimulates proinflammatory cytokines production by epithelial cells

polymorphonuclear leukocytes (Finck-Barbancon et al. 1993). Phosphorylation of LukS by protein kinase A was found to be required for the leukocytolytic activity (Muniesa et al. 2003; Brüssow et al. 2004). Very similar toxin genes were found at the same location in a morphologically and molecularly distinct *S. aureus* phage, SLT. *S. aureus* prophage PV83 also encodes a leukocidin, this time a *lukM-lukF* gene combination. The recent sequencing of several *S. aureus* strains confirmed and extended the observations from the phage-sequencing projects. Two phages are very similar between the two strains: N315 and Mu50A. The two prophages carry known virulence factors: a gene encoding enterotoxin P (the *sep* gene), a superantigen involved in the symptoms of food poisoning, and a gene encoding staphylokinase (the *sak* gene), suspected to be involved in the proteolytic destruction of host tissue. In addition, an M-like protein fragment is encoded by a gene preceding *sep*. The virulence genes flank the phage lysis cassette on both sides. However, the two prophages are not identical. Especially over the lysogeny and early genes, the two prophages differed in numerous small modular exchanges (Brüssow et al. 2004).

During infection, bacterial pathogens encounter the serum and phagocyte-mediated elements of the innate immune system. Staphylococci produce a number of proteins involved in phagocyte evasion, including a recently discovered chemotaxis inhibitory protein (CHIPS) that binds to and attenuates the activity of the neutrophil receptors for complement and formylated peptides. This function is proposed to protect *S. aureus* from neutrophil-mediated killing an important host defense against staphylococci. The gene encoding CHIPS (*chp*) has been shown to reside on a functional phage that also transduces the staphylokinase (*sak*) and enterotoxin A (*sea*) genes and eliminates hemolysin production, presumably by insertional inactivation Staphylococci also produce the phage-encoded Panton-Valentine leukocidin (PVL), a cytotoxin with direct activity against human phagocytes. Thus, by inhibiting phagocytosis (CHIPS) and by directly attacking phagocytes (PVL), two different phage gene products counteract phagocyte-mediated destruction of their staphylococcal hosts (Wagner et al. 2002).

S. aureus isolate, strain MW2, was sequenced (Baba et al. 2002). It differed from strain N315 by numerous insertions, deletions, and gene replacements. MW2 contains two prophages: Sa2 and Sa3. Sa2 resembles *S. aureus* phage 12 but also carries the *lukS* and *lukF* genes in a constellation identical to that phage in SLT. Sa3 closely resembles phage PVL over most of their genomes, but the two phages differed in their content of virulence genes. A comparison of the different *S. aureus* prophages revealed that the toxin genes are mobile DNA elements of their own and suggested that they are not stably associated with an individual prophage. Horizontal gene transfer has played a fundamental role not only in the evolution of *S. aureus* prophages but also in that of their hosts (Brüssow et al. 2004).

Clostridium botulinum

These bacteria are strictly anaerobic gram-positive bacteria, which are ubiquitous in the environment. Clostridia produce extremely resistant spores which sporulate under anaerobic conditions. *C. botulinum* strains were originally defined by their

ability to produce one of the closely related but antigenically distinct members (A, B, C1, D, E, F, or G) of the botulinum neurotoxin family. Human botulism is caused by the consumption of toxin-contaminated food. In other cases, the bacteria replicate within the human gut or sometimes in infected wounds, where they release the toxin in situ. Botulinum neurotoxins (BoNTXs) produced by *Clostridium botulinum* are among the most poisonous substances known.

Of the seven types of BoNTXs, genes for type C1 and D toxins (BoNTX C1 and D) are carried by bacteriophages. The gene for exoenzyme C3 also resides on these phages (Sakaguchi et al. 2005). Each type of BoNTX is produced as a large polypeptide and converted to a di-chain molecule composed of L and H chains by bacterial or host proteases. The H chain is responsible for the binding of the toxin to the presynaptic membrane and for the translocation of the L chain into the cytosol. The botulinum neurotoxins are expressed as ca. 150-kDa precursors lacking classical signal peptides (Brüssow et al. 2004). Of the seven types of BoNTXs, genes for type C1 (BoNTX C1) and type D (BoNTX D) are carried by bacteriophages, which were discovered in the early 1970s. These phages are categorized into three groups according to their conversion spectra: phages from strains C-Stockholm (C-ST) and C-468, those from strains D-1873 and C-203, and those from strains D-South African and D-4947. These groups differ also in antigenicity, although they share a similar morphology. BoNTX phages were later found to encode exoenzyme C3, an ADP-ribosyltransferase of GTPases. It is also known that the lysogeny of BoNTX phages is unstable (Sakaguchi et al. 2005; Brüssow et al. 2004). The botulinum neurotoxins A, B, and F are encoded in the chromosome, while G is plasmid encoded (Brüssow et al. 2004; Zhou et al. 1993), and C1 and D are encoded by prophages. The *C. botulinum* lysogens can be cured easily, and cultures of the C1 and D toxin-producing strains release significant amounts of phage (Brüssow et al. 2004).

Vibrio cholerae

Epidemics of cholera caused by toxigenic *Vibrio cholerae* belonging to the O1 or O139 serogroup are a major public health problem in many developing countries. The disease is an acute dehydrating diarrhea caused principally by the potent enterotoxin, cholera toxin (CT), produced by these organisms during pathogenesis (Faruque et al. 2001). Although *V. cholerae* is a human pathogen, aquatic ecosystems are major habitats of *Vibrio* species, which include both pathogenic and nonpathogenic strains that vary in their virulence gene content. Of the >100 known *Vibrio* serogroups, the two toxigenic serogroups "classical" O1 and O139 have been associated with epidemic cholera. The two human-pathogenic *V. cholerae* serogroups (O1 and O139) have evolved by sequential acquisition of two key fitness factors: the toxin-coregulated pilus (TCP) and cholera toxin (CT) (Hassan et al. 2010; Brüssow et al. 2004). Both are encoded by phages or phage-like elements (Waldor et al. 1997; Faruque et al. 2001; Brüssow et al. 2004).

In toxigenic *V. cholerae*, CT is encoded by a filamentous bacteriophage designated CTXΦ, which exists as a prophage in the bacterial chromosome. CTXΦ phage genome encodes the functions necessary for a site-specific integration system and

thus can integrate into the *V. cholerae* chromosome at a specific attachment site known as *att*RS, forming stable lysogens. A typical CTXΦ genome has two regions, core and the RS2. The 4.6 kb core region encodes CT as well as the functions that are required for the virion morphogenesis, whereas the 2.5 kb RS2 region encodes the regulation, replication, and integration functions of the CTXΦ genome. The A and B subunits of CT are encoded by two separate overlapping open reading frames. DNA sequence analysis has shown that the RS2 region consists of three open reading frames (ORFs) including *rstR*, *rstA*, and *rstB*, and two intergenic regions *ig1* and *ig2* (Waldor et al. 1997; Faruque et al. 2001; Brüssow et al. 2004). CT is expressed in the host intestine as a classical AB toxin. The B subunit of CT binds to enterocytes and transports the catalytic A subunit into the host cell cytoplasm. There, the A subunit triggers signaling cascades leading to rapid chloride and water efflux into the intestinal lumen, causing watery diarrhea, the hallmark of epidemic cholera (Faruque et al. 2001; Brüssow et al. 2004). CT might enhance bacterial survival in the intestine. Studies have confirmed that some naturally occurring nontoxigenic strains of *V. cholerae* are infected by CTXΦ and converted to toxigenic strains with epidemic potential (Waldor et al. 1997). TCP is critical for intestinal colonization (Merrell et al. 2002; Brüssow et al. 2004). It is a type IV bundle-forming pilus, whose major subunit (TcpA) was identified in a screen for secreted virulence factors which are coregulated with CT. TCP is expressed in the human intestine and belongs to the major antigens in human infections. The genetic element encoding TCP (also termed VPI for "*V. cholerae* pathogenicity island") has been described as the genome of a filamentous phage (VPIΦ or TCPΦ), but the phage nature has been disputed recently (Karaolis et al. 1999; Brüssow et al. 2004). Classical AB toxin occurs via type II secretion. The CT moron of CTX can be functional and provides a selective advantage only in vibrios. Only here are the proper regulators and transport systems available. Filamentous phage CTXΦ, which does not encode its own OM (outer membrane) pore, also requires one component of the *eps* system for its escape from the bacterium (Brüssow et al. 2004). The major pathogenic genes in *V. cholerae* are clustered in several regions of the *V. cholerae* chromosome and the structure of these pathogenic gene clusters indicates that these are capable of being propagated horizontally. The TCP pathogenicity island appears to be the initial genetic factor required for the origination of epidemic strains, since the cholera toxin-converting bacteriophage uses TCP as its receptor for infecting *V. cholerae* cells. Analysis of the structure of the TCP pathogenicity island suggests that this could be of phage origin or may be transferred by transducing phages (Faruque et al. 2001).

Corynebacterium diphtheriae

C. diphtheriae is a strictly human-adapted Gram (+) bacterium. It can cause local infections of the tonsils, pharynx, nose, and conjunctiva and systemic intoxications when the released toxin destroys the parenchyma of the heart, liver, kidneys, or adrenal glands. The diphtheria toxin (DT) is the major virulence factor of this pathogen, and the DT gene is carried by a family of closely related bacteriophages. Diphtheria toxin (DT) is a classical AB toxin. The A subunit of DT is an ADP-ribosyltransferase which covalently modifies the elongation factor EF-2,

thereby inhibiting chain elongation during protein synthesis (Brüssow et al. 2004; Zasada 2013). The symptoms are caused by diphtheria toxin (DT) encoded by the corynebacteriophage tox gene, the expression of which is downregulated by the chromosomally encoded diphtheria toxin repressor (DtxR) in an iron-dependent manner (Dinu et al. 2014; Zasada 2013). DtxR is a global metabolic regulator and binds to its DNA sequence targets as a homodimer after activation by divalent metal ions. DtxR is required for appropriate iron-dependent regulation of DT expression. Currently, at least 18 DtxR binding sites are known to occur in *C. diphtheriae*, and they affect the expression of about 40 genes. Studies conducted in the 1950s showed that non-lysogenic *C. diphtheriae* strains C4 and C7 become toxicogenic after infection with the tox^+ corynephage beta but not with the *tox*-lacking corynephage gamma. *C. diphtheriae* phages have been poorly investigated. Most toxigenic *C. diphtheriae* strains contain DNA sequences related to phage beta, but the *tox* gene was also found to be associated with the distinct phages δ and ω (Dinu et al. 2014; Brüssow et al. 2004; Zasada 2013).

Streptococcus pyogenes

S. pyogenes is a protean pathogen, and humans are its only reservoir. One-third of all humans are colonized with *S. pyogenes*. The bacteria are commonly found in the throat and on the skin. *Streptococcus pyogenes* is a multiply lysogenized organism whose phage constitutes 10% of the total genome and encodes a wide variety of putative and established virulence factors, including a large class of pyrogenic exotoxins (Broudy and Fischetti 2003). Recent comparative genomic studies have demonstrated that streptococcal bacteriophage represents the major variation (up to 71%) between strains of *S. pyogenes* and potentially account for the distinct disease pathologies associated with otherwise similar strains. In addition to modulating the virulence of organisms found within a common species of pathogenic bacteria, toxin-encoding phage produced by such pathogens have been shown to toxin convert both environmental and commensal bacteria, generating pathogenic Tox^+ microbes. Thus, bacteriophage represents key vectors for the dissemination of bacterial virulence and the conversion of bacteria from nonpathogenic to pathogenic (Broudy and Fischetti 2003; Brüssow et al. 2004). Various *Streptococcus* virulence factors are phage encoded. One of the best known is erythrogenic toxin A carried by phage T12. T12 is a prototypic temperate phage of group A streptococci that infects *Streptococcus pyogenes*, converting harmless strains into virulent ones (McShan et al. 1997). T12 carries a gene that encodes erythrogenic toxin A (SPE-A), also known as scarlet fever toxin A. The T12 genome is circular with total length of 36 kb. It is known that T12 integrates into *S. pyogenes* chromosome by site-specific recombination into the anticodon loop of a gene that encodes serine tRNA. The phage integrase gene (*int*) and the phage attachment site (attP) are located upstream of the *speA* gene in the phage genome. The bacterial attachment (attB) site is located at the 3′ end of the tRNA gene and has a sequence homologous to the phage attachment site. The coding sequence of the tRNA gene remains intact after integration of the prophage (McShan et al. 1997). The SPE-A toxin is known to damage plasma membranes of blood capillary endothelial cells found under the skin that

results in a red skin rash. Strains that produce SPE-A toxin are responsible for diseases like scarlet fever and streptococcal toxic shock syndrome (STSS).

Another well-known phage-encoded virulence factor is hyaluronidase encoded by bacteriophage-carried genes, *hylP* and *hylP2*. A single *Streptococcus pyogenes* strain can carry one of these genes or both. Streptococcal hyaluronidase is used as a spreading factor due to its ability to attack the hyaluronic acid present around host cells as a cementing substance. The hyaluronidase (HylP) carried by phage H4489A contains a series of 10 Gly-X-Y amino acid triplets, closely resembling the repeating sequences found in collagen. Since the bacteriophage hyaluronidase is found in isolates from patients suffering with rheumatic fever, it is possible that this collagen-like repeat could lead to the induction of antibodies which may cross-react with tissue collagen and result in a disease (Hynes et al. 1995).

Furthermore, some *Streptococcus* temperate phages are known to transfer resistance for antibiotics such as resistance to tetracycline, chloramphenicol, macrolides, and streptomycin most probably via generalized transduction (Wagner and Waldor 2002). Also, proteins PblA and PblB encoded by SM1 phage enable *Streptococcus mitis* to bind to platelets more efficiently (Bensing et al. 2001a). Those proteins are parts of the phage particle. However, they act as *S. mitis* surface proteins if SM1 is integrated into the bacterial genome. While not directly responsible for pathogenesis by this bacteria species, presence of this prophage in its genome facilitates colonization of a host. The exact mechanism of this phenomenon is still unknown (Bensing et al. 2001b).

Conclusions

Prophages are the essential part of the microbial life. They are able to colonize a host using sophisticated mechanisms, keep their presence in the host sometimes using even more sophisticated solutions, and contribute to the life together by fine-tuning host metabolism and by offering to a host a new ability. This synergy allows hosts to gain new abilities, colonize new ecological niches, or be more efficient, more aggressive, or just more versatile in those, which are already occupied by host. Sometimes, a one prophage too far, superbugs are being created. Even though, on the basis of literature surveys, they seem to be one of the dominant examples of phage-host synergy, it is very likely that they just attract our attention due to the urgent need to understand and counteract their ability to cause disease. They most probably constitute very small, but well-visible minority. The lysogenic conversion they provide to the host may show a wide range of different effects. The deadly payload they can deliver to augment host virulence is really impressive. These range from immune system evasion genes like *bor* in prophage λ (Barondess and Beckwith 1995) through enzymes capable of decomposition of body components facilitating invasion and acquisition of nutrients (e.g., Hynes et al. 1995) to the most-deadly poisons known to mankind (Brüssow et al. 2004; Sakaguchi et al. 2005).

Prophages were for a long time very useful model organisms for studying basic molecular biology mechanisms. As our knowledge progressed, it appeared that the

mechanisms being studied are very complicated and sublimed, and the first, simplistic models just scratched the surface of the real complexity of these processes. Such processes like lysis versus lysogeny decisions evolved to be complicated and to take into consideration many different aspects of host physiology (e.g., Słominska et al. 1999). Now it appears that prophages can communicate with their own kind and influence the decisions made by phages infecting a host (Erez et al. 2017; Abedon 2017). Once such a mechanism was discovered, it is only a matter of time before there are many more examples of similar ways of communication influencing the most important decision in temperate prophage life and the life of its host.

The fact that prophage interaction with the host may be important for both has been known for a long time (e.g., Dykhuizen et al. 1978), but the knowledge of how widespread this interaction is in nature is a relatively new discovery (e.g., Ventura et al. 2003a, b). Now in a metagenomic era, it is certain new discoveries are waiting for us on this subject.

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