Review

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Filamentous phages: masters of a microbial sharing economy

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Abstract

Bacteriophage ("bacteria eaters") or phage is the collective term for viruses that infect bacteria. While most phages are pathogens that kill their bacterial hosts, the filamentous phages of the subclass Inoviridae live in cooperative relationships with their bacterial hosts, akin to the principal behaviours found in the modern-day sharing economy: peer-to-peer support, to offset any burden. Filamentous phages impose very little burden on bacteria and offset this by providing service to help build better biofilms, or provision of toxins and other factors that increase virulence, or modified behaviours that provide novel motile activity to their bacterial hosts. Past, present and future biotechnology applications have been built on this phage–host cooperativity, including DNA sequencing technology, tools for genetic engineering and molecular analysis of gene expression and protein production, and phage-display technologies for screening protein–ligand and protein–protein interactions. With the explosion of genome and metagenome sequencing surveys around the world, we are coming to realize that our knowledge of filamentous phage diversity remains at a tip-of-the-iceberg stage, promising that new biology and biotechnology are soon to come.

Keywords filamentous phage; phage; procoat protein; secretin; Zot
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See the Glossary for abbreviations used in this article.

Introduction

Overview of phage diversity and their application in biotechnology

Phages come in diverse morphological forms and show huge diversity in their genome size, structure and sequences. They are the predominant biological entity on Earth, with the exponential acquisition in genome and metagenome sequence data now making estimates of their diversity and impact undeniable [1–4]. In addition to their impact on shaping bacterial communities including our own microbiomes, the knowledge acquired from phages of various families (Fig 1A) has provided immeasurable benefit to biotechnology.

Much within the toolbox of modern-day molecular biology derived from researchers working towards an understanding of bacteriophage lambda, a member of the Siphoviridae family of non-contractile tailed phages (reviewed in [5]). Phage T7 is a member of the Podoviridae family (Fig 1A), and the "Sequenase" reagent that revolutionized DNA sequencing is a slightly modified form of phage T7 DNA polymerase [6], while the phage T7 RNA polymerase system has proved to be a workhorse for heterologous protein expression as well as enabling some of the first demonstrations and applications of *in vitro* transcription systems [7].

The family Microviridae includes the phage phiX174 (Fig 1A), famed as the first genome sequenced by Fred Sanger [8]; as the model system Arthur Kornberg used to prove that DNA synthesized *in vitro* using purified enzymes generated a biologically active entity [9]; and as the paradigm in which Craig Venter showed that a genome created from synthetic oligonucleotides was both necessary and sufficient to produce biologically active entities [10].

The family Leviviridae includes bacteriophage MS2 (Fig 1A), a minimalist virus that encodes only four proteins and which infects *Escherichia coli*. The MS2 coat protein in conjunction with the hairpin sequence derived from the MS2 operator sequence forms the basis of a revolutionary system for live-cell imaging of specific RNAs in eukaryotic cells [11].

Four Siphoviridae prophages, φNM1 to φNM4, detected in the genome of the Gram-positive bacterium *Staphylococcus aureus* [12], led to studies in phage-based payload delivery: a small set of genes from the φNM1 phage were then used to create a packaging capsid for the delivery of a CRISPR/Cas9 system directed against *S. aureus*, and this phage therapy has been applied to kill MRSA, an antibiotic-resistant form of *S. aureus* [13].

The family Inoviridae includes phage sub-families with diverse structures and lifestyles (Fig 1A). One of these subgroups, the filamentous phages, includes species that can be longer than the bacteria that they infect, ranging in length from 800 nm to 4 μm (Fig 1B). Most of our understanding of the biology of filamentous phages comes from a group of closely related *Escherichia coli* phages called Ff filamentous phages. These phages, historically called M13, fd and f1, have 98% DNA sequence identity, and their replication mechanisms are identical. They were independently discovered in sewage

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Glossary

CRISPR	clustered regularly interspaced short palindromic repeats
Ff	collective term for near-identical phages M13, fd and f1
MRSA	methicillin-resistant *Staphylococcus aureus*
RF	replicative form
T2SS	bacterial type 2 secretion system
T3SS	bacterial type 3 secretion system
T4P	bacterial type 4 pili

samples in the early 1960s [14–17], and they will here be collectively termed *E. coli* Ff phages. One characteristic feature of Ff phages (and most filamentous phages) is that they do not lyse the host. Indeed, when isolated, they do not form typical lytic plaques on bacterial lawns, but rather opaque zones of reduced growth. Much of what we know about the biology of filamentous phages comes from experiments on these Ff phages, but the Ff phages are by no means representative of the vast diversity observed in the filamentous phage family.

Ff phages gave rise to one of the early cloning vectors for DNA sequencing (M13 sequencing: reviewed by [18]), and Ff served as the basis for phage-display protein–protein interaction screening and the maturation of protein, peptide, antigen or antibody libraries. Several features of Ff facilitated its use as the basis for phage-display technology: (i) assembly of these virions occurs without lysis of the *E. coli* host, allowing secretion of phage particles throughout the culture to the highest titres of any phage (up to 10^{13} virions per ml of culture); (ii) the capsid proteins are amenable to genetic fusion to other proteins, and thus the display of a foreign protein on the surface of the virion; and (iii) the Ff genome is small and easily modifiable, and most importantly, the modified genome is packaged into virions displaying corresponding modified capsid protein. This physical link between protein and corresponding DNA allows isolation of a desired protein/peptide/antigen/antibody from a library along with the gene encoding that specific variant [19], with sequential screening improving the affinity of the interactions (Fig 1C). In 2018, the Nobel Prize in Chemistry was awarded for advances in directed evolution, incorporating the work by Sir Gregory Winter and George Smith on phage-display in the directed evolution of new proteins, particularly directed at the production of antibody-based therapeutics [20–22].

Escherichia coli Ff: the archetypal filamentous phage

Structurally, the two best-studied filamentous phage virions are *E. coli* Ff and the *Pseudomonas* Pf1 phages. Both are ~6 nm in diameter, with the Ff phage virion approximately 1 μm long and the Pf1 virion approximately 2 μm long. For point of reference, *E. coli* cells are 2–3 μm long. The Ff phage filament has a helical structure with a fivefold rotational axis with a twofold screw axis (C_5S_2) termed Class I, and Pf1 phages have a more complex structure ($C_1S_{5.4}$) termed Class II (see [23] and [24] for detailed reviews). It had been thought that filamentous phages with larger genomes have longer virions. However, as more and diverse virions are studied, this generalization seems to be overly simplistic. Ff and Pf1 have similar genome sizes (6,408 and 7,349 bp, respectively), but the Pf1 filament is more than double the length of Ff. The lumen of the Ff filament has more positive charges per capsid subunit, and modifications to charged residues in the lumen do indeed seem to alter the DNA packing and therefore the virion length [23–28]. This suggests that both size and physicochemical properties matter when it comes to packaging phage genomes. The Ff virion is made up of numerous copies of five different proteins (Fig 1B). The major capsid protein pVIII forms the body of the phage, and its copy number is dependent on the length of the genome: an Ff phage has approximately 2,700 copies of pVIII per virion, calculated from the known quaternary structure and the length of Ff phages. pVIII is a small, α-helical protein, with the positively charged C-terminus in the core of the virion.

The minor capsid proteins cap either terminus of the virion. pVII and pIX cap the leading (emergent) terminus of the nascent virion, while pIII and pVI cap the terminal end. In Ff phages, the minor capsid proteins are present in an equimolar stoichiometry [29] of five copies per virion as evidenced in labelling pIII with ZnS quantum dots [30]. Taken together, each cap of the filamentous phage probably conforms to the fivefold symmetry of the major capsid filament—although there are no high-resolution data to confirm this assumption. This arrangement is not universal among filamentous phages, with the *Pseudomonas* Pf3 phage having only four capsid proteins.

In the minor capsid proteins, the N-terminal half of pIII forms two receptor-binding domains termed N1 (TolA binding) and N2 (pilus binding) [31], while the C-terminal domain interacts with the hydrophobic protein pVI. This pIII:pVI interaction is required for both the stability of the virion and the release of the nascent virion from the host cell [32–34]. The proteins pVII and pIX are both small and hydrophobic, with predicted α-helical structures. They form a protein complex which interacts with phage DNA, packaging the signal hairpin to initiate the assembly of the virion, and thereby forming a cap on the leading terminus of the nascent virion [35]. The specific roles played by these proteins within the phage life cycle are elaborated later in this review.

Filamentous phages influence the virulence of bacterial pathogens

Whereas other types of phages are pathogens of their bacterial host, killing the bacterium during egress, filamentous phages infecting *E. coli* are episomal replicating phages that impose only a modest burden on the host. Thus, their relationship is more one of cooperation, with the host providing for delivery of phages throughout the environment. The biology of these ride-share phages will be covered in detail in the final sections. To understand the benefits to the host bacterium, several well-studied phage–host scenarios are considered where the phage requires relatively little from its host, yet contributes significantly to its virulence and therefore its evolutionary fitness.

Phages that make Vibrio capable of cholera

The filamentous phages of *Vibrio* have garnered great attention as they are intimately linked to the evolution of toxigenic strains of *Vibrio cholerae*. The major means by which cholera is caused by *V. cholerae* is secretion of the cholera toxin: an oligomeric protein encoded by two genes that are carried by the temperate bacteriophage CTX. Cholera toxin provides an advantage to the bacterial host, as it promotes profuse diarrhoea in humans which results in

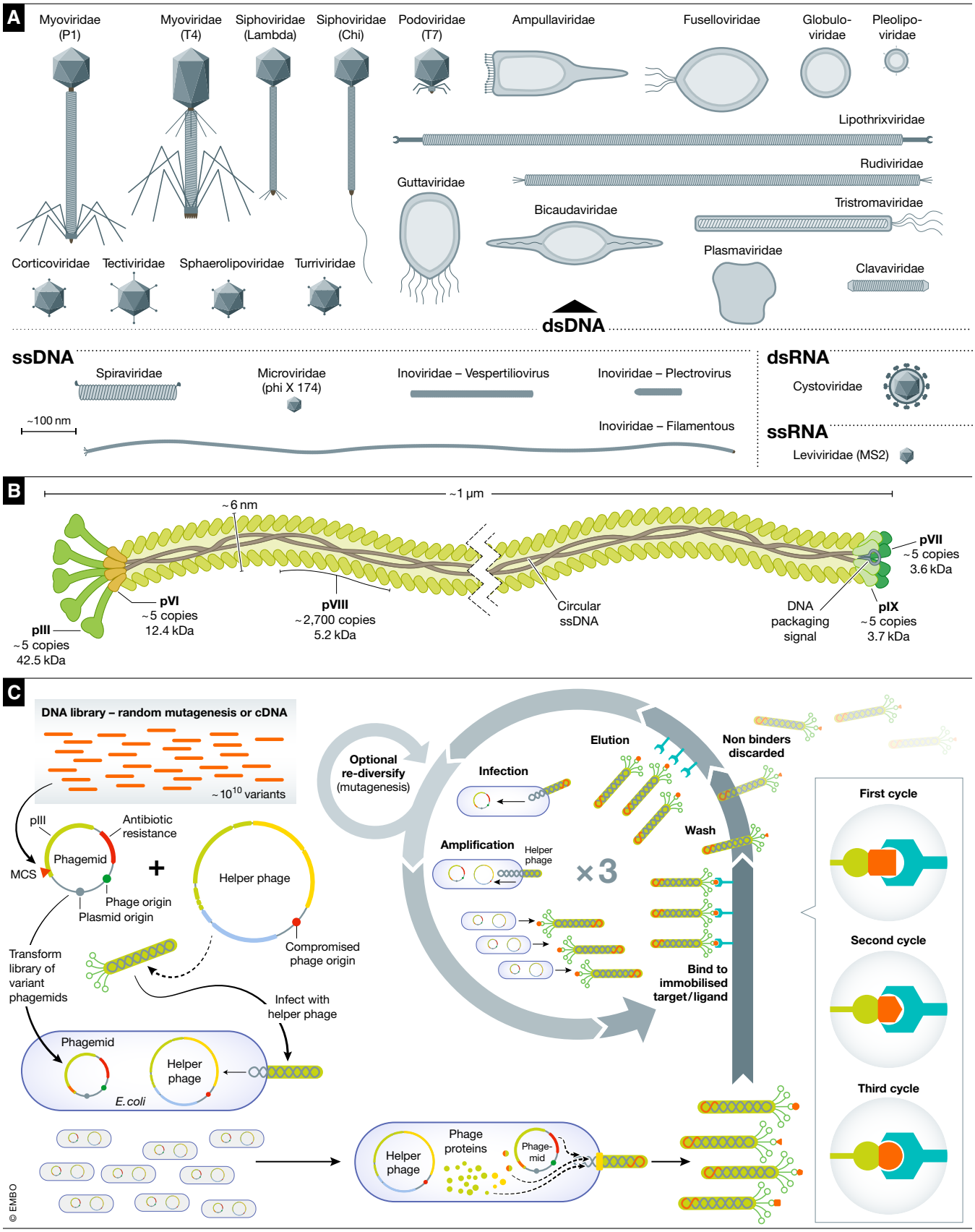

Figure 1.

Figure 1. Filamentous phages: classification and applications in biotechnology.

(A) Bacterial and Archaeal virus sub-families are represented and grouped based on their Baltimore classification. Relative sizes and symmetries are approximate. (B) Schematic representation of the *Escherichia coli* Ff phage showing the overall architecture and copy number of the structural proteins. (C) In phage-display screening, a natural or synthetic DNA library is cloned between the signal peptide and mature pIII-encoding gene on a phagemid vector containing an Ff origin of replication, a plasmid origin of replication, and a selectable marker. The phagemid pool is transformed into *E. coli* infected with a helper phage containing a compromised Ff origin. The helper phage produces all the machinery required for phage replication and assembly, and the phagemid produces modified pIII capsids. Phages are assembled and secreted with a subpopulation of the pIII capsid proteins containing the insert. The phage library them undergoes multiple rounds of "panning": (i) phages are applied to a matrix with immobilized ligand or target, those phages displaying peptides which bind to or are recognized by the target/ligand are bound while the non-binding phages are washed away, and then, (ii) bound phages are eluted and used to infect *E. coli* cells, which are then pooled and infected with a helper phage to amplify the library and produce phages for a subsequent round of panning. Multiple cycles of panning can produce peptides with increased affinity.

the dissemination of the pathogen. Infection of a non-pathogenic strain with CTXφ results in a toxigenic strain [36,37]. Furthermore, many other *Vibrio* phages and prophages interact with the CTXφ phage to promote the horizontal transfer of the cholera toxin genes (reviewed in detail by [36]). The host receptor for the CTXφ phage is the toxin-coregulated (TCP) type IV bundle-forming pilus, which itself is an important virulence factor. Controversially, the TCP pilus itself was previously described to be located on a large (40 kb) mobile element called VPI which was described as a filamentous prophage [38], but this was later shown to be incorrect; VPI is now regarded as an independent transmissible mobile genetic element, transferred via generalized transduction [39–42]. Recent assessment of prophages in diverse marine populations of *Vibrio* spp. suggests that filamentous prophages are numerous and widespread and play comprehensive roles in evolution through shaping niche adaptation and emergence of novel pathogenic strains emerging from environmental *Vibrio* communities [43–46]. Recently, a prophage with a similar genomic arrangement to CTXφ has been described in the human pathogen *Acinetobacter baylyi* (and *A. baumannii*) genome, though no homologous CTX toxin is present [47].

Phages that convince bacteria that altruistic death is a good thing

Pseudomonas aeruginosa harbours a group of related filamentous phages called the "Pf1-like" phages: Pf1, Pf4, Pf5 Pf6 and Pf7. The Pf1-like phages have been isolated from different strains of *P. aeruginosa* and are thought to be strain-specific variants of an ancestral prophage, with specificity now due to strain-specific variation of the type IV pili receptors [48,49]. These Pf1-like prophages are widespread among *P. aeruginosa* strains [48] and contribute to various aspects of host cell physiology. During biofilm growth, Pf4 genes are among the most upregulated with biofilm growth leading to the release of 100–1,000 times more phage virions [50]. This increase in expression of phage genes was also observed when *P. aeruginosa* was grown in anaerobic conditions mimicking a chronic late-stage cystic fibrosis lung [51], suggesting a cooperative role in the maintenance of infection. Pf4 plays a critical role in the overall structure, organized remodelling and seeding of mature biofilms. Filamentous phages present in the biofilm matrix self-organize into a viscous liquid-crystal-like arrangement providing the bacteria with increased surface attachment, and resistance to both desiccation and antibiotics [52]. Amidst biofilm microcolonies, a spatially and temporally organized Pf4-dependent cell death occurs leading to the remodelling and seeding of the biofilm.

Removal of the Pf4 prophage drastically reduces the development and stability of biofilm, which ultimately reduces virulence in mouse pneumonia model [53,54]. Furthermore, cells seeded from the Pf4-dependent remodelling of biofilms showed increased tendency to form "small colony variants", which showed higher levels of phage filaments on their surface and showed an increased attachment to surfaces and have been associated with pathogenicity [55].

The production of Pf4 has been linked to the maintenance of long-term chronic infections typical of *P. aeruginosa* infection. In a murine pneumonia model, phage production promoted a chronic lung biofilm infection, reducing the bacterial invasion of the host epithelial cells, and reducing the host inflammation and immune response to the infection. Combined with the increased antibiotic resistances observed in Pf4-producing biofilms, it seems the production of Pf4 by *P. aeruginosa* causes the bacteria to favour persistent infection over invasive infection which may be cleared by the animal host [52,56].

Phages that infect plant pathogens

Given the global uncertainty around food security, there is a major push for understanding how phages can shape microbial communities to disfavour bacterial pathogens on crop plants. The *Xanthomonas* phage Xf infects the rice leaf blight pathogen *Xanthomonas oryzae* and was one of the earliest filamentous phages described [57]. Beyond the initial physical description, little is known about this *Xanthomonas* phage. Other *Xanthomonas* phages, Cf, Cf1t/Cf1c and Cf16, were isolated from the citrus canker pathogen *Xanthomonas citri* in the 1980s. Cf1c was the first discovered example of a filamentous phage integrating into the host genome [58–60]. The Lf phage was later isolated from cruciferous vegetable black rot pathogen *Xanthomonas campestris* and was used as a cloning vehicle for the integration of DNA into *X. campestris*. Recently, the complete genome sequence of a "UK variant" of the Lf phage has been deposited in NCBI (GenBank: MH206184.1), along with four new *Xanthomonas* phages: Lf2, isolated form *Xanthomonas campestris pv. campestris*; Xv2, isolated from *Xanthomonas campestris pv. vesicatora*; Xf190, isolated from *X. oryzae*; and Xf409, isolated from *Xanthomonas oryzae pv. Oryzicola*; only XF109 has been described in the literature [61].

Screening another agricultural pathogen, *Ralstonia solanacearum*, revealed phages that can be loosely grouped into two families: the RSM1 and related phages [62]. These phages can integrate into their host genome and reside as prophages [63]. The RSM1-like phages (including RSM3, and Rs551) have been implicated in cooperatively trading away virulence to acquire drug resistance in a plant disease model. This the phage does by reducing *Ralstonia*'s production of extracellular polysaccharide, reducing twitching motility, increasing cell aggregation and reducing the expression of key virulence genes [64–70]. Given the phage modifies the host to ameliorate virulence, they have been proposed as biocontrol agents [64].

RSS1 phages increase the virulence of their *Ralstonia* host in a plant disease model, by increasing extracellular polysaccharide production, twitching motility, and the expression of some virulence genes [71]. It has been suggested that virulence-enhancing RSS1 phages are actually "superinfective" forms of the non-virulence-enhancing RSS0 prophage [63]. Prophage recombination on host chromosomes is common, and RS603 appears to be a hybrid phage with elements similar to the RSM1 and RSS1-like phages [72], whereas RS611 seems to be a hybrid of RSS1 and RSS0 [73].

Filamentous phages that impact marine microbial communities

In an era of climate change, where marine environments are under inarguable physical stress, filamentous phages isolated from marine bacterial species that shape their population structure are under intense investigation. The phage f327 was isolated from an arctic sea ice *Pseudoalteromonas* strain, and homologous phages to f327 are widespread through arctic sea ice. Carriage of the phage slows growth somewhat, but enhances the motility and chemotaxis of the host. This has been suggested as beneficial for the survival of the host in sea ice environments by preventing population "overblooming" during the relatively nutrient-rich summer period [74]. In the case of phage SW1 isolated from the deep-sea bacterium *Shewanella piezotolerans*, the phage genes and virion secretion are induced at low temperatures and presence of the phage results in differential transcription of many genes in response to low temperature and high pressure including flagella genes which results in a reduced motility [75–77]. A modified form of SW1 has been engineered into a low-temperature inducible expression vector for biotechnology applications [78]. Given the recent identification of filamentous phages from the hyperthermophile *Thermus thermophilus* [79], equivalent engineering could also generate high-temperature expression vectors for biotechnology applications.

Diagnostic features in filamentous phage genome sequences

With a few key exceptions, the currently described filamentous phages have been isolated from a small handful of species from the gammaproteobacterial genera *Escherichia* (and *Salmonella*), *Pseudomonas, Xanthomonas, Pseudoalteromonas, Yersinia, Shewanella* and *Stenotrophomonas*; and from the betaproteobacterial genera *Ralstonia*, and *Neisseria* (Table 1). With the benefit of a substantial body of work that defines the structural and regulatory genes that contribute to filamentous phage biology, genome sequences can now be mapped and studied comparatively (Fig 2A).

Across the various filamentous phage lineages, only the pI proteins stand out as being highly conserved and fully diagnostic (Fig 2B). The pI proteins have a conserved Zot domain (Pfam PF05707 Zot domain) at their N-terminus. This domain was named for the pI homologue in the Vibrio CTXφ phage called the Zonula Occludens Toxin, which is essential for the assembly and export of CTXφ phage virions, and has been implicated in the virulence of pathogenic *V. cholerae* strains by increasing intestinal permeability through binding to tight junctions (zonula occludens) between small intestine epithelial cells [80,81]. While the toxin itself carries the conserved Zot domain in its N-terminus, the Zot domain is not the toxic component: the C-terminal domain is cleaved from the Zot protein, and it is this non-conserved, C-terminal fragment that intoxicates the human epithelial cells [82–86]. Unfortunately, the "Zonula Occludens Toxin" automated annotation that has now attached itself to entries for most filamentous phages has resulted in incorrect assumptions about toxin activity in newly annotated phage genomes [87,88].

Phylogenetic analysis of the conserved pI proteins shows that they provide a basis for phage classification (Fig 3). The phylogeny also demonstrates that based on the current ICTV threshold for the classification of filamentous phage genera, there are many other clades that could be subject to future classification. In the simplest sense, grouping filamentous phages based on their pI proteins leads to distinct clades. For example, the *Escherichia* phages Ff, I22, IKe and If1, the *Vibrio* phages fs2 and VFJ phages group together as a single clade. This clade contains the phage genera *Saetivirus*, *Inovirus* and *Lineavirus*, and all the members are non-integrative episomal phages. A second clade is formed by the *Vibrio* VfO36K/f237, VCY and KSF1 on one branch and a group containing the *Pseudomonas* "Pf1-like" phages (Pf1, Pf4, Pf5) and the *Vibrio* CTXφ phage on another branch. All the *Neisseria* prophages are in a large diverse third clade with the *Xanthomonas* Cf1c-like and Xf109/Xf409 phages; the *Stenotrophomonas* phages SMA6, SHP2 and PSH1; the *Ralstonia* RSS1 family phages (RSS1, RSS0, p12J, PE226); and the *Pseudomonas* episomal phage Pf3. A fourth clade is formed with the *Ralstonia* "RSM1-like" phages (RSM1, RSM3, RS603) of the genera *Habenivirus*, the *Stenotrophomonas* phages SMA7 and SMA9 and the *Xanthomonas* phages Lf and Lf2 on one branch, and the Yersinia/Escherichia CUS phages (CUS-1, Ypf/CUS-2) on a separate branch. A clade encompassing the *Fibrovirus* genus contains the *Vibrio* phages fs1, VSK, VSKK, VEJ, VGJ and Vf33, as well as the *Shewanella* phage SW1. A more distantly related branch contains the *Thermus* phages OH3 and OH16 and the Gram-positive *Propionibacterium* phage B5.

Filamentous phage life cycle: infection

Almost all of the described filamentous phages infect Gram-negative hosts, and thus must traverse two membrane barriers. While the cell surface receptor is unknown for the majority of described filamentous phages, where it is known the receptor has been shown to be a pilus of some description. For example, Ff and related phages use the F sex/conjugation pilus (Fig 4A); various *Vibrio* spp. phages use either the toxin-coregulated type 4 pili or the mannose-sensitive haemagglutinin type 4 pili; *Pseudomonas* phages Pf1 and Pf3 use the PAK type 4 pili and conjugative RP4 pilus, respectively; *Xanthomonas* phage Cf uses an uncharacterized type 4 pilus; and *Acinetobacter* phage CRAφ utilizes a competence pilus [47,89–93]. While commonly referred to as "pili", the F-pilus and the various type 4 pili are evolutionally, mechanistically and structurally unrelated; the common feature important for phage entry into its bacterial host seems to be simply the retractile function of the pilus.

Docking onto the host cell pilus is mediated through the pIII protein (Fig 4A). Electron microscopy observations suggest that the phages bind to the tip of the pilus, in a tip-to-tip fashion [94]. This has been experimentally confirmed in the case of CTXφ, where pIII binding to the pilus tip subunit (called the minor pilin, TcpB) of the toxin-coregulated type 4 pilus in *Vibrio cholerae* has been directly demonstrated [95]. It is speculated that binding of the phage to the pilus induces signalling to retract the pilus, bringing the phage towards the host cell surface [96]. Upon pilus retraction, the pIII

Table 1. Filamentous phages

Phage	Host	Additional Information #	References
Ff	*Escherichia coli*	*Inovirus* ICTV type species. AKA M13, f1, fd. Host receptor—F "sex" pilus. Non-integrative episomal replication. 900 × 6 nm	[195]
If1	*Escherichia coli*	Unassigned genus. Host receptor—I pilus. Non-integrative episomal replication. 900 × 6 nm	[196]
IKe	*Salmonella typhimurium* and *Escherichia coli*	*Lineavirus* ICTV type species. Host receptor—N or I_2 pilus. Non-integrative episomal replication. 1,000 × 6 nm	[197–199]
I22	*Escherichia coli*	*Lineavirus*. Host receptor—N, I_2 or P pilus. Non-integrative episomal replication. 1,000 × 6 nm	[199,200]
CUS-1	*Escherichia coli*	Prophage. RF observed but no RF sequence available. Associated with pathogenic strains. Imparts slight fitness advantage to host during mammalian infection—unknown mechanism	[168,169]
Ypf/CUS-2	*Yersinia pestis*	Prophage. Very similar to CUS-1. RF observed but no RF sequence available. Associated with pathogenic strains. Imparts slight fitness advantage to host during mammalian infection—unknown mechanism. 1,200 × 8 nm	[170,201]
CTXφ	*Vibrio cholera*	Unassigned genus. Host receptor—toxin-coregulated type IV bundle-forming pilus. Associated with pathogenic strains. Encodes cholera toxin A/B. Typically requires a satellite prophage or prophage duplication to produce infective phage virions.	[36,202,203]
RS1	*Vibrio cholera*	Satellite prophage depends on CTXφ, KSF1 or VJG.	[36,204,205]
fs2	*Vibrio cholera*	*Saetivirus* ICTV type species. Non-integrative episomal replication.	[191,206]
TLC	*Vibrio cholera*	Satellite prophage depends on fs2. Promotes the integration of CTXφ.	[37]
VFJ	*Vibrio cholera*	*Saetivirus*. Non-integrative episomal replication. Looks like a mosaic of fs2 and VEJ or VCY. Host shows inhibition of flagellum formation and had slightly increased antibiotic resistance through an unknown mechanism. 1,400 × 7 nm	[207]
VCY	*Vibrio cholera*	Unassigned genus. Integrates into host genome. Associated with environmental *Vibrio* isolates. 1,800 × 7 nm	[44]
KSF1	*Vibrio cholera*	Unassigned genus. Host receptor—MSHA type IV pili. 1,200 × 7 nm	[208]
VfO3K6	*Vibrio parahaemolyticus*	Unassigned genus. AKA f237 or pO3K6. Episomal replication. Associated with pathogenic strains. 2,500 × 8 nm	[74,209]
VfO4K68	*Vibrio parahaemolyticus*	Unassigned genus. Derivative of VfO3K6 potentially carrying novel toxin gene. Episomal replication 1,300 × 6 nm	[74]
Vf33	*Vibrio parahaemolyticus*	Unassigned genus. AKA Vf12. Integrates in to host DNA. 1,400 × 7 nm	[210]
fs1	*Vibrio cholera*	*Fibrovirus* ICTV type species. Host receptor—MSHA type IV pilus. Integrates into host genome. 1,000–1,200 × 7 nm	[191,211]
VSK	*Vibrio cholera*	Considered a variant of fs1	[191]
VSKK	*Vibrio cholera*	Considered a variant of fs1	[191]
VEJ	*Vibrio cholera*	Considered a variant of fs1. Host receptor—MSHA type IV pilus. Recombination with CTXφ allows horizontal transfer of cholera toxin genes.	[184,191]
VGJ	*Vibrio cholera*	*Fibrovirus*. Host receptor—MSHA type IV pilus. Integrates into host genome. Recombination with CTXφ allows horizontal transfer of cholera toxin genes. 1,000 × 7 nm	[89,185]
Pf1	*Pseudomonas aeruginosa* (PAK)	Unassigned genus. Episomal replication Host receptor—PAK type IV pili. Virion inhibits Candida albicans growth via sequestering iron. 2,000 × 6 nm	[91,212,213]
Pf3	*Pseudomonas aeruginosa*	Unassigned genus. Episomal replication. Host receptor—RP4 conjugative pilus. Non-integrative episomal replication. 700 × 6 nm	[91,214]
Pf4	*Pseudomonas aeruginosa* (PAO1)	Prophage. Integrates into host genome. RF observed but no RF sequence available. Implicated in host virulence via biofilm remodelling and dispersal mediated by host cell death; and the formation of virulent small colony variants (SCV). Virion inhibits *Aspergillus fumigatus* metabolism and Candida albicans growth via sequestering iron. Predicted length 37,000 × 6 nm	[53,55,213,215]
Pf5	*Pseudomonas aeruginosa* (PA14)	Prophage. Integrates into host genome. RF observed but no sequence available.	[216]
Pf6	*Pseudomonas aeruginosa* (PAO1-MPAO1)	Pf4 variant from the PAO1-MPAO1 strain. Inserted at different locus to Pf4 and containing two additional genes encoding protein kinases. AKA RGP42.	[49,217]

Table 1 (continued)

Phage	Host	Additional Information #	References
RSM1	*Ralstonia solanacearum*	*Habenivirus* ICTV type species. Host receptor—probably type IV pili. Integrates into host genome. Decreases host virulence. Increases host cell aggregation. 1,400 × 10 nm	[68–70,178,191]
RSM3	*Ralstonia solanacearum*	*Habenivirus.* Host receptor—probably type IV pili. Integrates into host genome. Decreases host virulence, growth rate, extracellular polysaccharide production, motility, and expression of some virulence genes. Increases host cell aggregation and antibiotic resistance. Proposed biocontrol agent.	[64,65,68]
RS603	*Ralstonia solanacearum*	*Habenivirus.* Only RF episomal form described (lacks integrase from RSM1/3). Appears to be a hybrid of RSM1/3 and RSS1/0. 1,120 × 8 nm	[72]
RS551	*Ralstonia solanacearum*	Not classified (probably *Habenivirus*). Decreases host virulence, extracellular polysaccharide production, motility. Integrates into host genome. 1,200 × 7 nm	[66,67]
RSS1	*Ralstonia solanacearum*	Unassigned genus. Host receptor—probably type IV pili. Increases host virulence, extracellular polysaccharide production, motility and expression of some virulence genes. May be an episomal "superinfective" form of RSS0. 1,100 × 10 nm	[68–71,178]
RSS0	*Ralstonia solanacearum*	Not classified. Very similar to RSS1 with additional ORF encoding potential DNA-binding regulator and an *attP* site.	[62]
RS611	*Ralstonia solanacearum*	Not classified. Appears to be a hybrid of RSS1 and RSS0 with a deletion of two ORFs. 1,120 × 8 nm	[73]
p12J	*Ralstonia pickettii*	Not classified. Unclear if the deposited sequence is a phage or prophage sequence	[218]
PE226	*Ralstonia solanacearum*	Not classified. Only RF episomal form described. 1,050 × 6–9 nm	[87]
Xf109	*Xanthomonas oryzae*	Unassigned genus. Integrates into host genome. 1,210 × 8 nm	[61]
Xf409	*Xanthomonas oryzae*	Not classified. similar to Xf109	*
Lf	*Xanthomonas campestris*	Not classified. Complete genome of "UK variant" available. Suggested to be integrative, though not conclusively demonstrated. 1,000 × 8 nm	[219]
Lf2	*Xanthomonas campestris*	Not classified	*
Xv2	*Xanthomonas campestris*	Not classified	*
Xf	*Xanthomonas oryzae*	Not classified. No sequence information. 977 × 8 nm	[57]
Cf	*Xanthomonas citri*	Not classified. No sequence information. 1,000 nm long	[220]
Cf1t	*Xanthomonas citri*	Not classified. Similar to Cf. Integrates into host genome	[58,59]
Cf1c	*Xanthomonas citri*	Unassigned genus. Variant of Cf1t. Forms clear plaques. Sequence available.	[221]
Cf16	*Xanthomonas citri*	Not classified. Integrates into host genome	[222]
XacF1	*Xanthomonas citri*	Not classified. Integrates into host genome. Lowers host EPS production, motility, and growth. Host shows reduced virulence in plant disease model. 600 nm long	[223,224]
MDAφ	*Neisseria meningitidis*	Not classified. AKA Nf1-A. Host receptor—probably type IV pili. Integrates into host genome. Presence of prophage correlates with hypervirulent invasive strains. Increases bacterial host attachment to epithelial cells. 1,200 nm long	[177,225]
Nf1	*Neisseria meningitidis*	Not classified. Prophage	[171]
Nf2	*Neisseria meningitidis*	Not classified. Prophage	[171]
Nf3	*Neisseria meningitidis*	Not classified. Prophage	[171]
Nf4	*Neisseria gonorrhoeae*	Not classified. Prophage	[171]
Ngo6	*Neisseria gonorrhoeae*	Not classified. Virus derived from synthetic phagemid containing the Nf4-G2 prophage. Reported to infect diverse proteobacterial species.	[226]
CRA	*Acinetobacter baylyi*	Not classified. Prophage. RF observed but no RF sequence available. Host receptor—probably competency pilus. Phage inhibits natural competency of cells	[47]
SHP1	*Stenotrophomonas maltophilia*	Unassigned genus. AKA PSH1. Only episomal RF reported. 2,100 × 15 nm	[227]
SHP2	*Stenotrophomonas maltophilia*	Not classified. Only episomal RF reported. 800 × 10 nm	[228]
SMA6	*Stenotrophomonas maltophilia*	Unassigned genus. Integrates into host genome	[229]

Table 1 (continued)

Phage	Host	Additional Information #	References
SMA7	*Stenotrophomonas maltophilia*	Unassigned genus. Integrates into host genome	[229]
SMA9	*Stenotrophomonas maltophilia*	Unassigned genus	[88]
f327	*Pseudoalteromonas* sp. BSi20327	Not classified. AKA pSM327. Only RF described. Decreases host growth rate. Increases motility and chemotaxis. Widely distributed in arctic sea ice samples. 1,500 × 14 nm	[230]
SW1	*Shewanella piezotolerans*	Not classified. Integrates into genome. Phage replication and genes expression induced at low temperatures. Seems to have a role in flagella regulation.	[76,77,231]
OH3	*Thermus thermophilus*	Unassigned genus. Only RF episomal observed. 830 × 8 nm	[79]
OH16	*Thermus thermophilus*	Not classified. Like OH3 but with an additional transposase. Only genome sequence—no description	*
PH75	*Thermus thermophilus*	Not classified. Only protein sequence of major capsid protein reported.	[232]
B5	*Propionibacterium freudenreichii*	Unassigned genus. Only RF episomal form described 620 × 12 nm	[233]
CAK1	*Clostridium beijerinckii*	Not classified. Only RF observed. Infectivity not demonstrated. No genome sequence. 1,000 × 5–8 nm	[234]
NP-2014	Environmental	DNA sequenced during human virion project from amniotic fluid—classed as "*Ralstonia* phage" though no rational for this naming is given	*
WW-nAnB	Environmental	DNA isolated from raw sewage. Previously detected in faecal samples but incorrectly described as "non-A, non-B hepatitis"	[235]

This should not be considered a complete list of filamentous phages/prophages. * No literature publicly available, only genome sequence available. # Virion sizes are as reported in relative literature. Different methodologies may result in difference in measurements (particularly with respect to the width measurements).

containing terminus of the virion is thereby brought into the periplasmic space, to engage a secondary receptor in the periplasm of the host. It seems most likely that this entry event would be through the pilus pore, and the spatial constraints of the pore would allow this. In the cases investigated so far, the secondary receptor is always the inner-membrane-anchored protein TolA, which extends out into the periplasm (Fig 4A). TolA is a component of the TolQRA complex, a nanomachine element in the "Tol-Pal" system that controls membrane integrity and invagination during cell division [97–100]. While this primary function of TolA may not be relevant to phage entry, the primary function of TolA is essential; thus, the TolQRA secondary receptor is highly conserved, hence ever available for filamentous phage infection [101].

Upon pilus binding and retraction, the N2 domain of protein pIII appears to have a crucial role in assisting the infection process. An experiment where the N2 domain of pIII was recombinantly expressed in host bacteria prevented F-pilus extension and locked the pilus in a retracted state through an unknown mechanism [102], and it has been proposed that this allows the phage to securely traverse the outer membrane and disassemble into the inner membrane without F-pilus extension or subsequent infection with other phages interfering with the process [19]. Once the N1 domain of pIII is uncovered and brought into the periplasm via the retraction of the pilus, it binds to the C-terminal domain "III" of TolA. Beyond the pIII-TolA binding event, very little is known of subsequent infection steps or how the virion DNA traverses the host cell inner membrane. The extreme C-terminus of pIII is predicted to contain a transmembrane α-helix and two short amphipathic α-helices which are essential for phage infection, and pIII has been shown to have pore-forming properties in artificial lipid bilayers [103], suggesting that the C-terminus of pIII inserts into the membrane creating a pore that could allow phage DNA access to the host cytoplasm. The major capsid proteins end up embedded in the inner membrane, with their N-termini on the periplasmic side of the membrane, potentially reused for packaging new phage particles [104–106], suggesting there is some kind of ordered disassembly of the virion capsid at the inner membrane that drives the phage DNA into the host cytoplasm.

Filamentous phage life cycle: genome replication

For Ff phages, episomal replication of the ssDNA genome (Fig 4B) is a well-characterized process and early experiments on Ff genome replication provided seminal information for our current understanding of rolling circle DNA replication, a mechanism relevant to understanding bacterial plasmid replication, the amplification of various virial genomes, and the replication of mitochondrial DNA in at least some species of eukaryotes [107,108].

As shown in Fig 4B, the filamentous phage genome is injected into the host cell cytoplasm as single-stranded circular DNA referred to as the infective form (IF). Replication of the phage genome is largely controlled by the intergenic sequence (IG) located between the gIV and gII genes, which contain the + and – strand origins of replication and the packaging signal that all form double-stranded hairpins in the ssDNA genome. Filamentous phage genome replication is entirely dependent on the core bacterial DNA replication machinery. Upon entry, the host RNA polymerase σ70 holoenzyme binds to the – strand origin hairpin, which mimics a bacterial −35 and −10 promoter sequence, with an affinity much higher than a typical bacterial promoter [109,110]. RNA polymerase begins to synthesize RNA on the ssDNA template but stalls and backtracks at

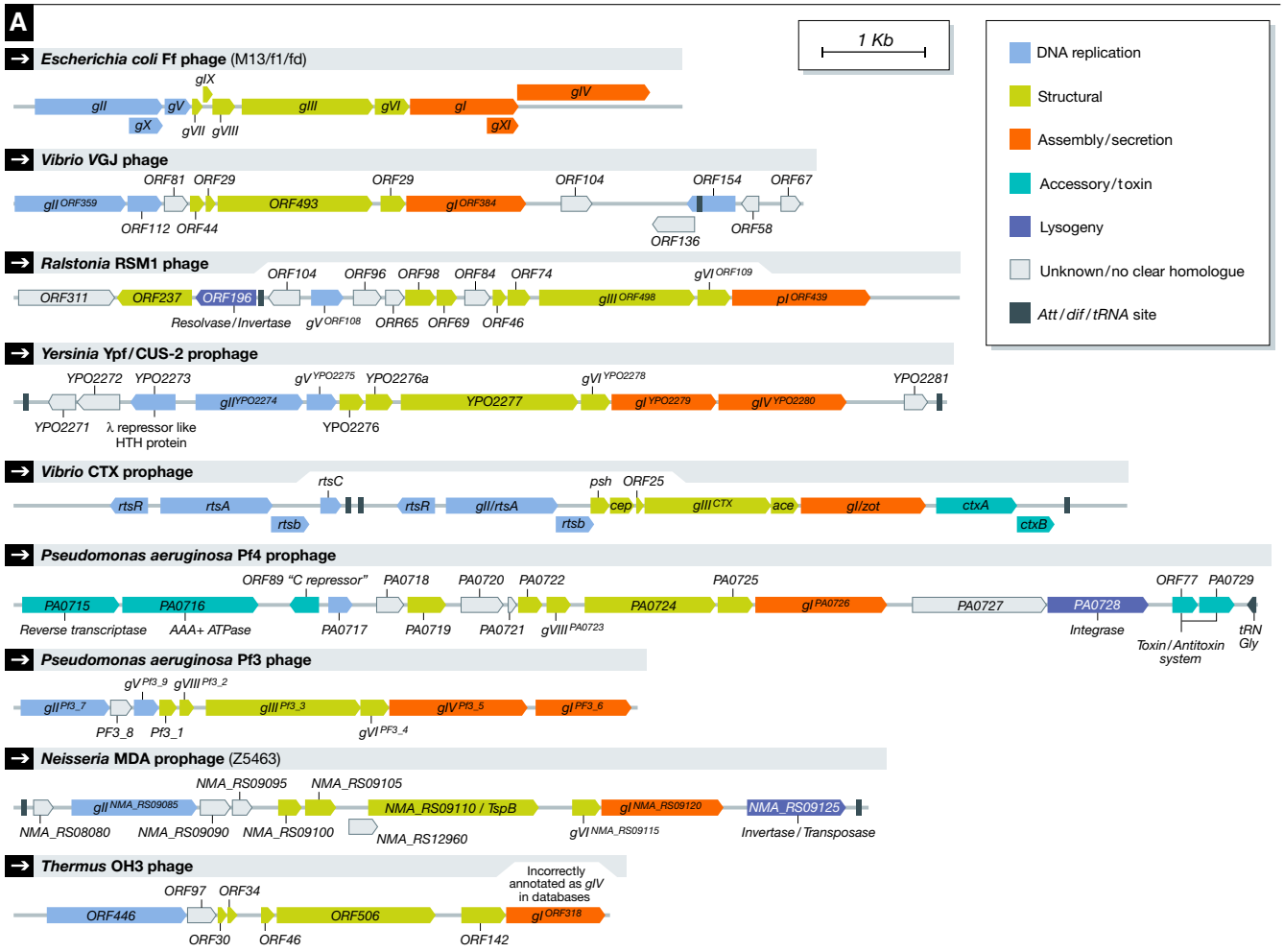
A
1 Kb
DNA replication
Structural
Assembly/secretion
Accessory/toxin
Lysogeny
Unknown/no clear homologue
Att/dif/tRNA site
Escherichia coli Ff phage (M13/f1/fd)
gII gV gX gVII gVIII gIII gVI gI gIV gXI gIX
Vibrio VGJ phage
gII ORF359 ORF81 ORF29 ORF112 ORF44 ORF29 ORF493 gI ORF384 ORF104 ORF154 ORF136 ORF67 ORF58
Ralstonia RSM1 phage
ORF311 ORF237 ORF196 Resolvase/Invertase ORF104 gV ORF108 ORF96 ORR65 ORF98 ORF69 ORF84 ORF46 ORF74 gIII ORF498 gVI ORF109 pI ORF439
Yersinia Ypf/CUS-2 prophage
YPO2271 YPO2272 YPO2273 λ repressor like HTH protein gII YPO2274 gV YPO2275 YPO2276 YPO2276a YPO2277 gVI YPO2278 gI YPO2279 gIV YPO2280 YPO2281
Vibrio CTX prophage
rtsR rtsA rtsb rtsC rtsR gII/rtsA rtsb psh cep ORF25 gIII CTX ace gI/zot ctxA ctxB
Pseudomonas aeruginosa Pf4 prophage
PA0715 Reverse transcriptase PA0716 AAA+ ATPase ORF89 "C repressor" PA0717 PA0718 PA0719 PA0720 PA0721 PA0722 gVIII PA0723 PA0724 PA0725 gI PA0726 PA0727 PA0728 Integrase ORF77 Toxin/Antitoxin system PA0729 tRN Gly
Pseudomonas aeruginosa Pf3 phage
gII Pf3_7 PF3_8 gV Pf3_9 Pf3_1 gVIII Pf3_2 gIII Pf3_3 gVI Pf3_4 gIV Pf3_5 gI Pf3_6
Neisseria MDA prophage (Z5463)
gII NMA_RS09085 NMA_RS08080 NMA_RS09095 NMA_RS09090 NMA_RS09105 NMA_RS09100 NMA_RS09110 / TspB NMA_RS12960 gI NMA_RS09120 gVI NMA_RS09115 NMA_RS09125 Invertase / Transposase
Thermus OH3 phage
Incorrectly annotated as gIV in databases
ORF446 ORF30 ORF97 ORF34 ORF46 ORF506 gI ORF318 ORF142
B
pI / Zot
pII/ pX
pIII
pV
pVII
pIV
pVI
pIX
pVIII
Sequence similarity
Less similar
More similar
Number of identical proteins
1 4
Enterobacteria Ff (M13)
Pseudomonas Pf3
Vibrio CTX
Neisseria MDA
Pseudomonas Pf4
Yersinia Ypf
Vibrio VGJ
Thermus OH3
Ralstonia RSM3
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Figure 2.

Figure 2. Diversity of filamentous phage genomes.

(A) Schematic representation of filamentous phage genomes: for each gene identified in the genome, the putative function is noted either based on experimental evidence, inferred from sequence homology, or based on conserved domain predictions. Scale bare represents genome size in nucleotide base pairs. (B) Protein sequence similarity network plot of all predicted open-reading frames from 56 filamentous phage genomes. The great proportion of orphan proteins in this plot demonstrates that at the protein sequence level, there is a very high degree of diversity in filamentous phages. Each circle node represents a sequence, and each connecting line represents a BLAST score better than 1e-5. Identical proteins are collapsed into one circle with the size representing the number of proteins denoted. Representative species are coloured as shown, and the identity of the Ff proteins is annotated in the plot.

a section of the genome and dissociates leaving a short RNA primer (18–20 nt long) which is extended by the host DNA polymerase III holoenzyme to generate the – strand of the genome and the double-stranded replicative form (RF) of the phage genome [111,112]. The RF can also be synthesized from other regions of the genome but with drastically lowered efficiency [113]. The RF is further processed by the host gyrase to form a supercoiled RF [114,115].

There are three crucial aspects to the initial function of the RF. Firstly, it serves as a template for the transcription of the initial mRNA transcripts encoding the phage proteins including pII and pX, which are required for the amplification of the phage genome. Secondly, it serves as a template for the replication of the RF. Thirdly, it serves as a template for replication of the IF. Amplification of the RF and IF through rolling circle replication is mediated by the phage protein pII.

Protein pII is a strand transferase which binds to the newly synthesized supercoiled RF at the + strand origin of replication, cleaves the + strand and attaches to the 5′ end [115–117]. The free 3′ end can now serve as a primer for the host DNA polymerase holoenzyme to synthesize a new + strand, displacing the original + strand as it progresses. In Ff, these pII-mediated steps are also dependent on the host replicative Rep helicase and integration host factor, whereas some *Vibrio* and *Pseudomonas* Pf phages use the hosts alternate DNA repair helicase UvrD and the histone-like HU proteins [118–120]. Once the replication completes a full circle, pII cleaves and cyclizes the free ends resulting in a ssDNA IF and dsDNA RF [116]. Early in the infection cycle, the IF is converted into new RF, as described above, until approximately 50 RF copies are present in the host [121], whereas the RF can serve as a template for further rolling circle replication generated IFs and as a template for the transcription of phage mRNA transcripts. As the number of RFs templated for transcription increases, so too does the number of phage proteins present in the host cell. Late in the infection when the level of pV protein reaches a critical number, it forms dimers and binds at the bottom of the hairpin formed by the packaging signal in the IF DNA and begins to coat the entire length (except the packaging signal) of the DNA, with one antiparallel ssDNA strand bound by each side of the dimer forming a long helical DNA–protein complex containing approximately 1,600 copies of pV [122,123]. pV also plays a role in timing the infection cycle in the host and coordinating the level of IF for packaging. Late in the infection, the increasing levels of pV directly inhibit both the synthesis of the negative strand and the translation of the pII and pX proteins, which results in the accumulation of IF DNA [124,125]. Although pII inhibition is dispensable for a successful infection cycle [126], pX protein—which is identical to the C-terminal third of pII and translated from an internal start codon within the pII gene—appears to play an additional, though unclear, regulatory role in the levels ration of IF and RF [127,128].

Despite this seemingly costly exercise in DNA replication by the phage, it really is a cooperative use of host resources. Most filamentous phages are produced rapidly in an initial stage lasting less than 10 host cell generations, with each bacterial cell producing around 200 phages per generation, after which time the host settles into a stable state where the phage genome is only replicated at very low levels and very few phages are produced [121]. By contrast, within a few minutes of infection the classic *E. coli* tailed phage T4 converts the host cell into a factory, with the sole purpose of producing phage particles. The T4 genome contains more than 270 genes, with many of them encoding the machinery required for the hostile takeover of the host cell. T4 immediately inhibits host DNA replication, transcription and translation and completely remodels the host metabolism to favour phage production. The host DNA is degraded and recycled into phage DNA. Within 20–30 min, the host is actively lysed and 100–200 T4 virions are released (for a review of T4 host interactions, see [129]).

Filamentous phage life cycle: phage egress

In contrast to tailed phages, the physical dimensions of filamentous phages would presumably prevent their assembly within the cell; as such, the virion is assembled at the bacterial cell envelope, with the maturing phage actively secreted through the cell envelope in a non-lytic manner. The single-stranded DNA genome appears to lack any significant Watson–Crick base pairing apart from a hairpin at one terminus, called the packaging signal [24]. The newly replicated ssDNA IF genome is coated with the DNA-binding protein pV in the cytoplasm, and this serves to stabilize and expose the "packaging signal" (Fig 4B and C) that will target the DNA–protein complex to the inner membrane of the host cell (Fig 4C). The trans-envelope export complex is comprised of two phage proteins: pI and pXI (pXI is translated from an internal start site in the pI gene and is identical to the transmembrane and C-terminal periplasmic third of pI). These two phage proteins, pI and pXI, each have a single transmembrane domain to anchor them to the host cell inner membrane, and thereby form one half of a secretion complex, equivalent but not homologous to a bacterial type 2 secretion system. Intriguingly, the translocation channel across the outer membrane utilized by filamentous phages *is* a protein of the secretin protein family, referred to as pIV (Fig 2B), with the secretin protein family also forming the exit channel of type 4 pili as well as type 2 secretion systems. The phage secretion machinery is remarkable in its apparent simplicity; whereas the T2SS and type 4 pili require many periplasmic or envelope proteins to assemble their pilus, the filamentous phage secretion machinery assembles a more complex DNA–protein hybrid filament with only two (or three) proteins.

The pI protein is embedded in the membrane by a signal anchor domain, leaving the N-terminal ~250 residues in the bacterial

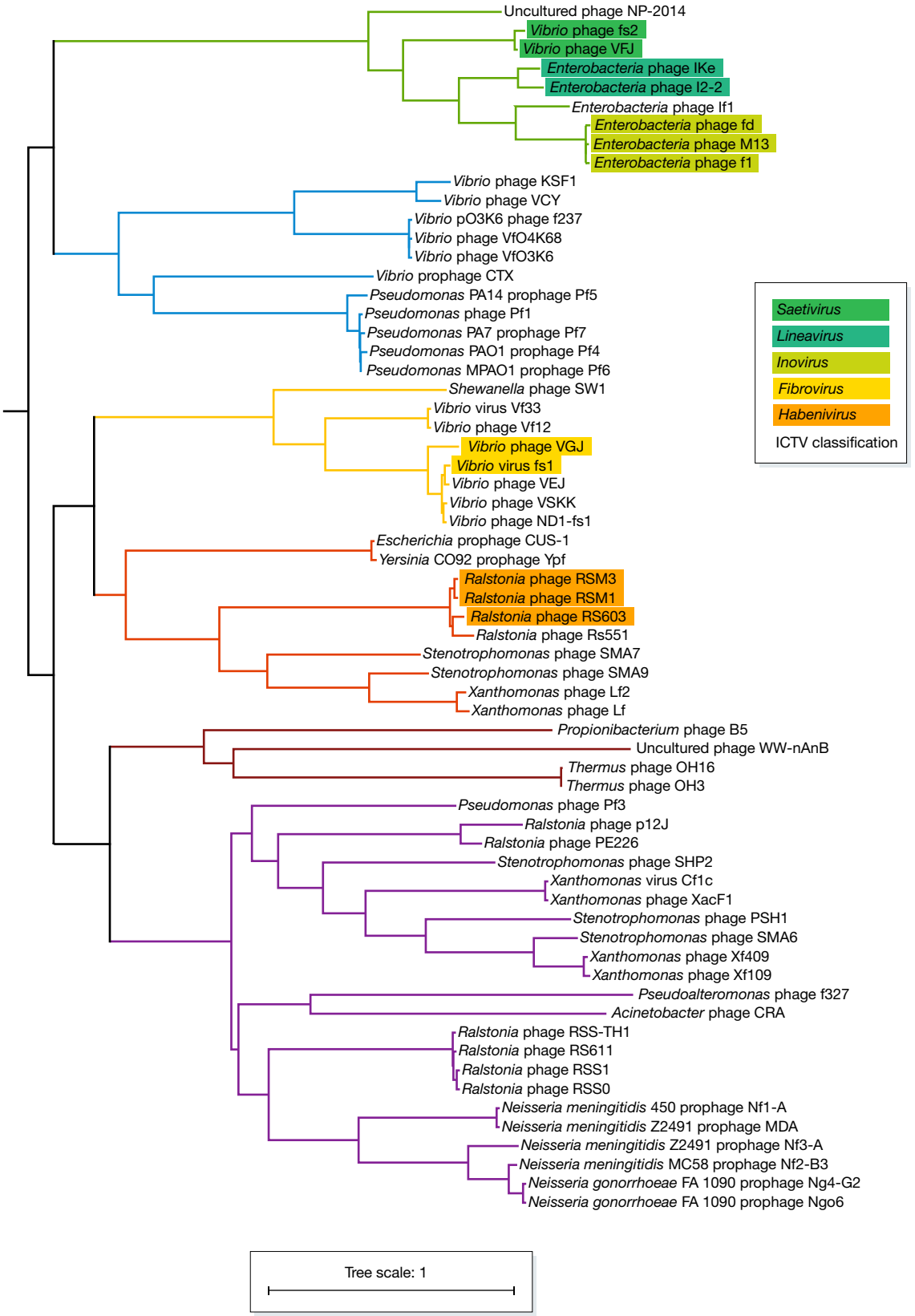

Figure 3.

Figure 3. Phylogenetic tree of filamentous phages.

Phylogenetic tree built of the conserved pI homologues of known filamentous phages and prophages. Alignments were calculated with mafft generated (L-INS-i option), and sites for tree inference chosen using trimal (automated1). The tree was calculated with RAxML "PROTGAMMAAUTO" criteria (final model LG) and "autoMRE" bootstrap convergence test and midpoint rooted [236–238]. Clades are coloured as described in the text, and leaves are coloured based on their ICTV genera classification.

cytoplasm and the C-terminal ~80 residues in the periplasm, and the signal anchor domain of pXI results in the remainder of the protein exposed to the periplasm. Being (initially) signal-anchored proteins, both pI and pXI require the bacterial Sec machinery for insertion into the membrane [130]. The functional benefit of pXI to the phage is unclear, and while both pI and pXI are essential for Ff phage export [131,132], the internal start site generating protein pXI is not a conserved feature. The cytoplasmic domain of pI is predicted to act as an ATPase, powering the assembly and transport of the phages through the envelope. This is supported by the finding that Ff phage assembly is dependent on ATP hydrolysis [133].

pI and pXI form a complex with the outer membrane protein pIV in the absence of other phage proteins or DNA, suggesting that this is a genuine secretion nanomachine representing a pre-initiation step of phage morphogenesis [134]. Like other secretins, pIV resides in the outer membrane [130], though the mechanism of secretin assembly into the outer membrane remains unknown [135]. Low-resolution cryo-electron microscopy of purified pIV showed that the protein forms ring-like structures with an outer ring diameter of approximately 13.5 nm and an inner (channel) diameter of approximately 6 nm. This channel diameter would be just sufficient to allow passage of the filamentous phage particle, which has a diameter of 6 nm. Although no clear symmetry could be observed, nanogold labelling suggested that there are 14 subunits per secretin complex [136]. From recent near-atomic resolution cryo-electron microscopy models of secretins from the type 2 secretion system [137–139] and previous mutagenesis experiments on the pIV gate regions [140], we can extrapolate some information about the structure and function of pIV. Secretins form homo-oligomeric complexes comprised of 15 copies of the secretin subunit. The outer membrane channel is formed by extended β-strands (four from each subunit) with two β-hairpins folded upwards into the lumen of the barrel to form a β-barrel cone forming an internal gate. A recent structure of the T3SS with the gates in an open state showed that the two β-hairpins forming the internal gate straighten and move approximately 40 Å upwards against the outer β-barrel [141]; these are the same regions identified as "leaky" gate mutants in a mutagenesis screen of pIV [140]. Thus, it is plausible that the pIV secretin gate would open in a similar fashion during phage filament extrusion.

While some phages encode their own secretin (pIV), most do not (Fig 2B). Nonetheless, it seems likely that secretin-mediated egress is a common feature for the filamentous phages, given two well-studied cases where phages were shown to share the host cell secretion for their own travel out of the bacterial cell. When CTXφ is secreted from *V. cholerae*, it uses the endogenous T2SS secretin EspD for phage secretion, and this process is independent of the T2SS inner membrane machinery [142,143]. When MDAφ infects *N. meningitidis*, it uses the endogenous type 4 pilus secretin PilQ for phage secretion [144]. It is assumed that the majority of other filamentous phages lacking a dedicated secretin use a similar mechanism. This ride sharing by filamentous phages contrasts with the process of host cell protein secretion, wherein secretins normally require highly organized interactions with their cognate inner membrane machinery [145–147]. This suggests that evolution has driven phage pI proteins to present periplasmic domains that mimic and/or displace secretin-binding domains of the cognate bacterial secretion systems.

The Ff structural proteins are initially integrated in to the bacterial IM prior to assembly. In a past era of pioneering work on how bacteria target and assemble membrane proteins, filamentous phage coat proteins were used as models and helped drive our understanding of membrane protein biogenesis in *E. coli* [148–152]. The "procoat protein" pVIII has a signal sequence to engage with the Sec/YidC machinery of the host cell, and was therefore used as a model protein to dissect the role of the targeting pathways and membrane translocation events in *E. coli* [149,153,154]. The phage proteins also have sequences predicted as transmembrane domains; initially perplexing, since the phage contains no lipids, it is now clear that the coat proteins use a mode of integration into the inner membrane in order to coalesce together and to co-translocationally displace the pV (DNA-binding protein) from the ssDNA, and thereby coat the DNA to create the filamentous phage capsid [23]. How the coat proteins are extracted out of the inner membrane during virion assembly is unknown, but an active area of investigation (see also Box 1).

Box 1: In need of answers

(i) The structure of both termini of the Ff virion—Although various methods have resolved the super structure of the virion shaft and several crystal structures of domains of the minor capsid pIII exist, the tertiary and quaternary structures of the pVII:pIX and pIII:pVI caps and how they interface with the helical shaft remain unknown. Modern advances in electron microscopy may lead to advanced in our understanding of the virion structure.

(ii) Assembly of the virion—How do the capsid proteins transition from their inner membrane embedded to their structural virion forms? How are they extracted from the membrane, and what initiates the assembly? Is the assembly reaction actively driven by the pI ATPase, akin to the pilus of the type 4 pili?

(iii) Egress through the bacterial OM—do all filamentous phages use secretins for secretion across the OM? Do those lacking an endogenous OM secretin pores all hijack the bacterial secretion systems to exit the cells as seen in CTXφ and the T2SS in *V. cholerae*?

(iv) The "origin" of filamentous phages and bacterial secretins—The origin of filamentous phages is directly linked to the evolution of secretins and thus the T2SS, T3SS and the T4P. So, are filamentous phages the progenitors of bacterial secretin systems or simply hijacking established systems?

(v) How widespread are filamentous phages in nature? In the current review, we have provided evidence suggesting filamentous prophages are distributed widely throughout the bacterial and archaeal kingdoms, though only very few have been experimentally validated. A more systematic approach to identifying filamentous phages and prophages both experimentally and in bacterial and metagenomic sequence data will elucidate our understanding of these fascinating systems.

Filamentous phage assembly is initiated by the minor capsid proteins pVII and pIX which are small hydrophobic proteins, integrated into the bacterial inner membrane. These proteins bind to the exposed packaging signal of the phage DNA, thereby forming the cap of the virion to initiate assembly of the emergent phage [35,155–157]. During the early stages of phage infection, the major capsid protein pVIII becomes one of the most abundant proteins in the cytoplasm with upwards of 4 million copies per

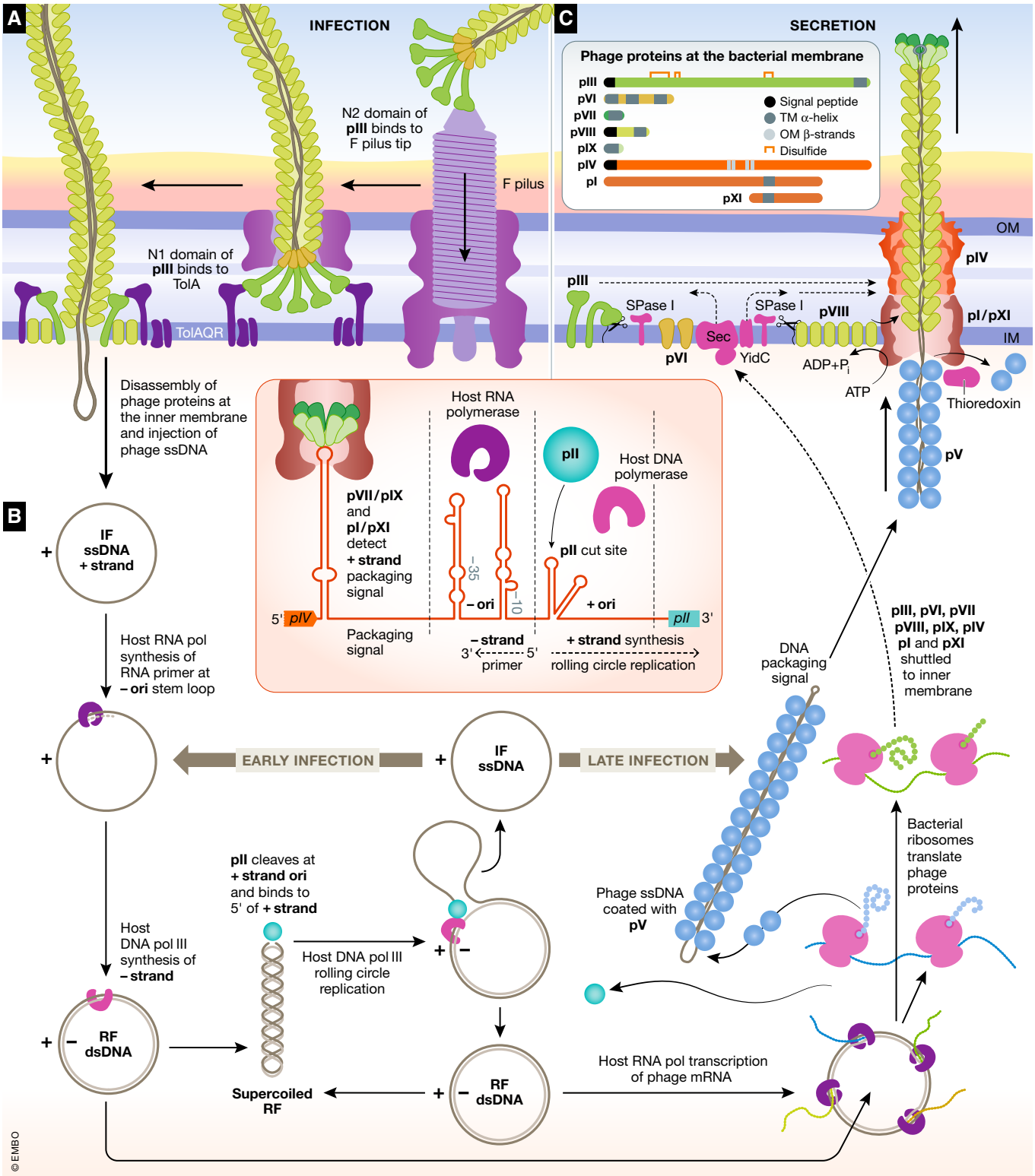

Figure 4.

Figure 4. Lifecycle of the archetypical filamentous Ff phage.

(A) In the initial stage of phage attachment, the N2 domain of pIII binds to the tip of the F-pilus on the surface of the bacterial cell. Upon F-pilus retraction, the pIII/pVI terminus of the phage would be brought into periplasm of the host cell. The N1 domain of pIII binds to the host protein TolA in the TolQRA complex in the inner membrane. The next stage, which has not been characterized, would need to result in phage disassembly and injection into the cytoplasm of the ssDNA genome termed the "infective form" (IF). (B) Phage replication ensues through recruitment of the host RNA polymerase to a hairpin at the negative (−) origin of replication, resulting in synthesis of a short RNA primer. The positive (+) strand is then extended by the host DNA polymerase III, generating a double-stranded phage genome termed the "replicative form" (RF). Early in the infection, this can serve as a template for host RNA polymerase to generate phage mRNA, to be translated into phage proteins. The phage protein pII binds to the + origin of replication and nicks the + strand, and the resulting 3′ end is extended by host DNA polymerase III displacing the "old" + strand. Upon one full cycle, pII cleaves and ligates the + strands resulting in a single-stranded IF and a double-stranded RF. The RF can then undergo multiple rounds of rolling circle replication to replicate the phage genome and also serve as a template for transcription and translation of phage proteins. Later in the infection, single-stranded IF is coated by phage protein pV, leaving the packaging signal-free in preparation for secretion. (inset) A schematic representation of the phage intergenic region containing the packaging signal, the − origin of replication and the + origin of replication is shown. (C) Structural phage proteins and phage proteins required for assembly and secretion are shuttled to the inner membrane and processed by the SecYEG, YidC and signal peptidase machinery. The packaging signal hairpin of the pV-coated ssDNA is bound by the minor capsid proteins pVII and pIX and recognized by the pI/pXI IM assembly proteins. As the emerging ssDNA traverses the inner membrane, pV is removed and replaced by the membrane-embedded major capsid protein pVIII. As pVIII is added to the emerging phage, the tip is forced outwards through the oligomeric secretin-gated channel pIV. The terminal phage capsid proteins pIII and pVI detect and cap the end of the nascent phage allowing its release from the host cell. Host proteins are represented as various shades of purple. Phage proteins involved in DNA replication and packaging are represented by shades of blue. Phage proteins involved in secretion are shown as shades of orange. Structural phage proteins are shown as shades of green. (inset) Phage proteins which interact with the bacterial membranes are shown. Topogenic signal peptides and transmembrane regions are annotated.

cell [121]. The protein may (e.g. *E. coli*) or may not (e.g. *P. aeruginosa*) contain an N-terminal signal peptide, but its integration into the inner membrane is mediated by the YidC translocon [158–161]. The signal peptide of pVIII is cleaved by the host cell signal peptidase, leaving the membrane-embedded capsid with its N-terminus in the periplasm and the C-terminus in the cytosol [149,150,162]. Accumulation of the local concentration of pVIII capsid protein in the inner membrane leads to protein oligomerization [163,164] driving phage filament assembly (Fig 4C). Precisely how the assembly of the coat protein subunits is mediated is unknown, but models for the transition of membrane-embedded pVIII into virion filaments have been proposed and reviewed recently [23,165].

Once the entirety of the phage DNA is coated in the major capsid protein, it must be released from the inner membrane and capped with the minor capsid proteins pIII and pVI, which form a stable complex at the base of the virion [166]. If pIII of pVI is deleted, the virion cannot be released from the host cell and the filament continues to grow integrating multiple copies of the phage DNA into the growing filament [32]. As well as being responsible for host cell receptor binding and injections of the phage DNA during the infection process, pIII plays a key role in the release of mature phages. pIII is targeted to the inner membrane via its N-terminal signal peptide, which is then cleaved leaving pIII embedded in the membrane via a C-terminal transmembrane helix [33,167]. The C-terminal domain of pIII is implicated in binding to and thereby releasing the newly assembled phages. It is thought significant rearrangements must occur in pIII to achieve this, but how the pIII/pVI capping complex detects the end of the phage and induces this final release of the virion is not known [32].

Prophages and discovery science

Many filamentous phages can integrate into the host chromosome and be replicated along with the bacterial genome during cell division. In addition to providing clues as to the details of phage–host interactions, this feature means that filamentous phages can be discovered through genome and metagenome surveys of diverse environments. Filamentous prophage integration (Fig 5 and Table 2) can be mediated by one of two methods: using host recombinases XerC and XerD or using a phage-encoded recombinase.

Integrated prophages pay their way through contributions to virulence and other advantageous phenotypes. An *E. coli* prophage called CUS-1 is correlated with invasive extraintestinal pathogenic *E. coli* strains, and the prophage encodes *puvA,* which was identified as contributing to bacterial virulence in a rat disease model [168,169]. An almost identical phage, Ypf/CUS-2, has been described in *Yersinia pestis*, where again the prophage is associated with virulent plague strains and disruption of the prophage resulted in reduced virulence in mice [170].

Many prophages thought to encode filamentous phages have been described in *Neisseria gonorrhoeae* and *Neisseria meningitidis* strains [144,171–173]. Whole-genome sequence analysis of four *Neisseria* species found 12 complete prophages and 11 incomplete prophages. These various prophage elements have been implicated in plasticity of *Neisseria* genomes, and assisting the massive chromosomal rearrangements observed between strains [171,173–175]. For *N. meningitidis*, the presence of one of these prophages, Nf1-A, has been assigned Meningococcal Disease Associated (MDA) because it was one of the only loci correlated with hypervirulence [144,176]. It was later shown that carriage of the prophage does not increase the virulence of the septicaemic phase of the disease in a human tissue disease model, but that phage secretion increases biofilm formation and colonization of epithelial cells [177]. In this scenario, the MDAφ virion behaves analogously to type IV pili, with many virions remaining associated with the bacterial surface and promoting bacteria–bacteria interactions.

Our current understanding of prophage integration into bacterial chromosomes

The host-mediated XerC and XerD site-specific recombination is the most well-characterized integration process, because of studies focused on understanding the method of integration in *Vibrio* filamentous phages. The endogenous role of the recombinases XerC and XerD is to ensure the segregation of two bacterial circular chromosomes during genome replication, by catalysing recombination between two *dif* (deletion-induced filamentation) resolving dimeric chromosomes formed during DNA replication [178]. Filamentous phages have hijacked this system by containing a *dif*-like site (termed *attP*) on their genome (the bacterial chromosome site for integration, which is typically a *dif* site is termed the *attB* site) (Fig 5).

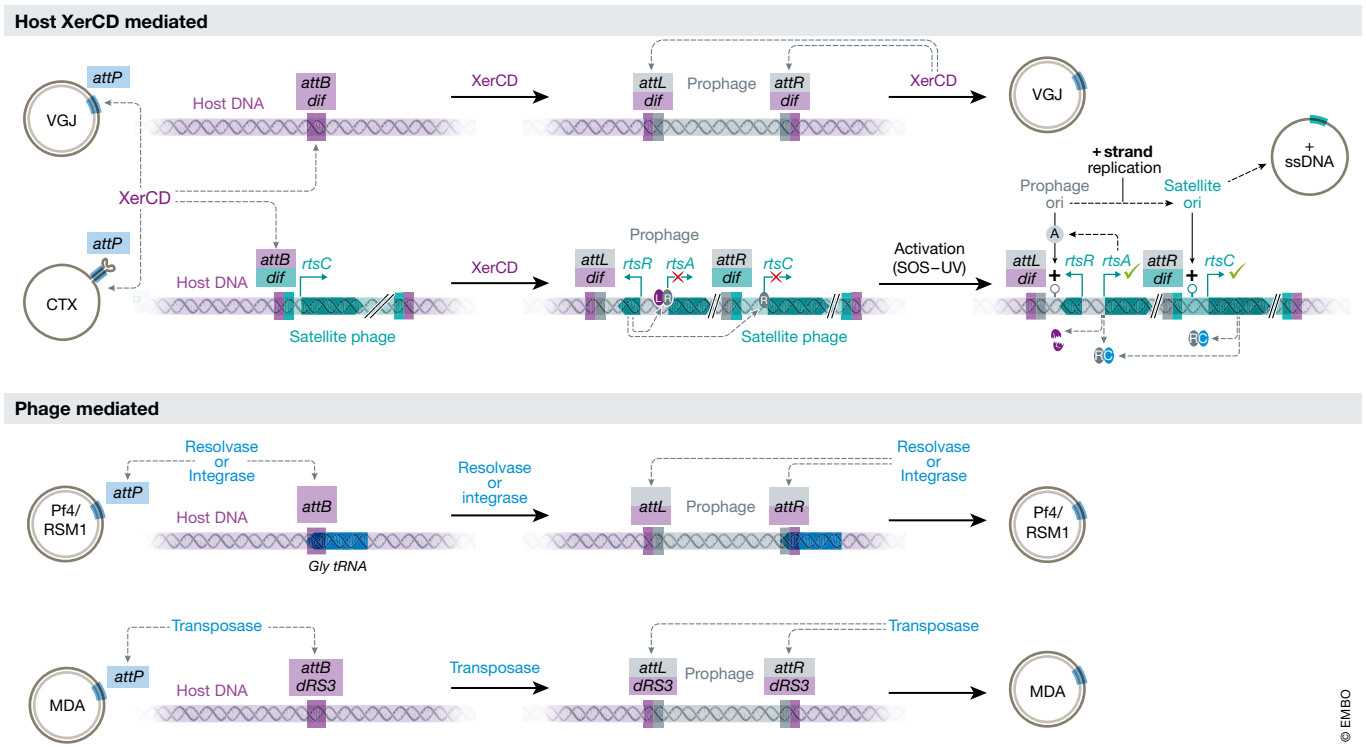

Figure 5. Methods of filamentous phage host chromosome integration and excision.

Methods for filamentous prophage integration into the host chromosome are shown. Top: Host-mediated (XerCD) integration via two methods. Vibrio phage VJG uses a reversible integration—a dsDNA RF phage genome with an *attP* site is recognized by the host XerCD recombinase which mediates homologous recombination at the *dif* site on the host chromosome. The prophage can be excised by XerCD-mediated recombination at the resulting *attL* and *attR* sites. Vibrio phage CTXφ uses an irreversible integration—XerCD recognizes an *attP* site formed by a hairpin in the ssDNA phage genome and mediates homologous recombination at the *dif* site on the host chromosome (and typically a satellite phage). Due to nature of the *attP* hairpin, the resulting *AttL* site on the prophage is defective and thus cannot be excised by XerCD. Replication of the resulting prophage is inactivated by a regulatory loop involving the phage-encoded repressor RtsR (R), the host repressor LexA (L) and the satellite activator RtsC (C). Upon activation by the host SOS response, LexA is degraded and the positive regulator RtsC is produced and binds to the RtsR repressor allowing expression of the phage replication protein RtsA (functionally equivalent of pII). RtsA binds to the + ori on the prophage genome and acts in an analogous way to that of pII on RF circular DNA. The resulting phage ssDNA is amplified and packaged as described in Fig 4B. In examples of phage-mediated integration (bottom), *Pseudomonas* phage Pf4 uses a phage-encoded integrase to reversibly integrate itself into the Gly tRNA site of the host chromosome and Ralstonia phage RSM1 uses a phage-encoded resolvase to reversibly integrate into Ser tRNA site on the host chromosome, while Neisseria phage MDAφ uses a phage-mediated transposase to integrate at a 20-bp repeat region (dRS3) on the host chromosome.

Table 2. Filamentous prophage integration methods

Integration method	Host integration site *attB*	Examples[a]
Host XerC/XerD (reversible)	*dif*	VGJ, TLC (satellite), VEJ, VSK, Vf33, fs2, Lf, Cf1c, Cf16, RSS0
Host XerC/XerD (irreversible)	*dif*	CTX, CUS-1, Ypf
Integrase—tyrosine recombinase	Gly tRNA (Met-tRNA)	Pf4, Pf5, Pf7 (Pf6)
Resolvase—serine recombinase	Ser tRNA	RSM1, RSM3
Transposase	Variable—dRS3 repeat	MDAφ

[a]This is not a complete list of phages using the listed method. Not all experimentally validated (may be based on *attP* sequence homology or presence of recombinase on phage/prophage genome).

The *Vibrio* phage VGJ genome contains an *attP* site, which XerCD can bind to in the dsDNA RF of the phage genome and catalyse recombination between the *attP* and *attB* sites. This integration is reversible because the prophage is flanked by functional *attL* and *attR* sites [179,180]. However, the *Vibrio* phage CTXφ genome contains two adjacent "defective" *attB* sites in an inverted orientation. In the ssDNA IF, these sites can form a forked hairpin that results in a functional *attB* site. XerCD can catalyse recombination with the bacterial *attB* site [181,182]. Integration of the CTXφ ssDNA template leaves a Holliday junction intermediate that must be resolved by the host DNA replication machinery through an unclear process. The prophage cannot be excised as the *att* hairpin cannot form on the chromosome. Thus, once CTXφ has been integrated as a prophage, it must undergo rolling circle replication on the

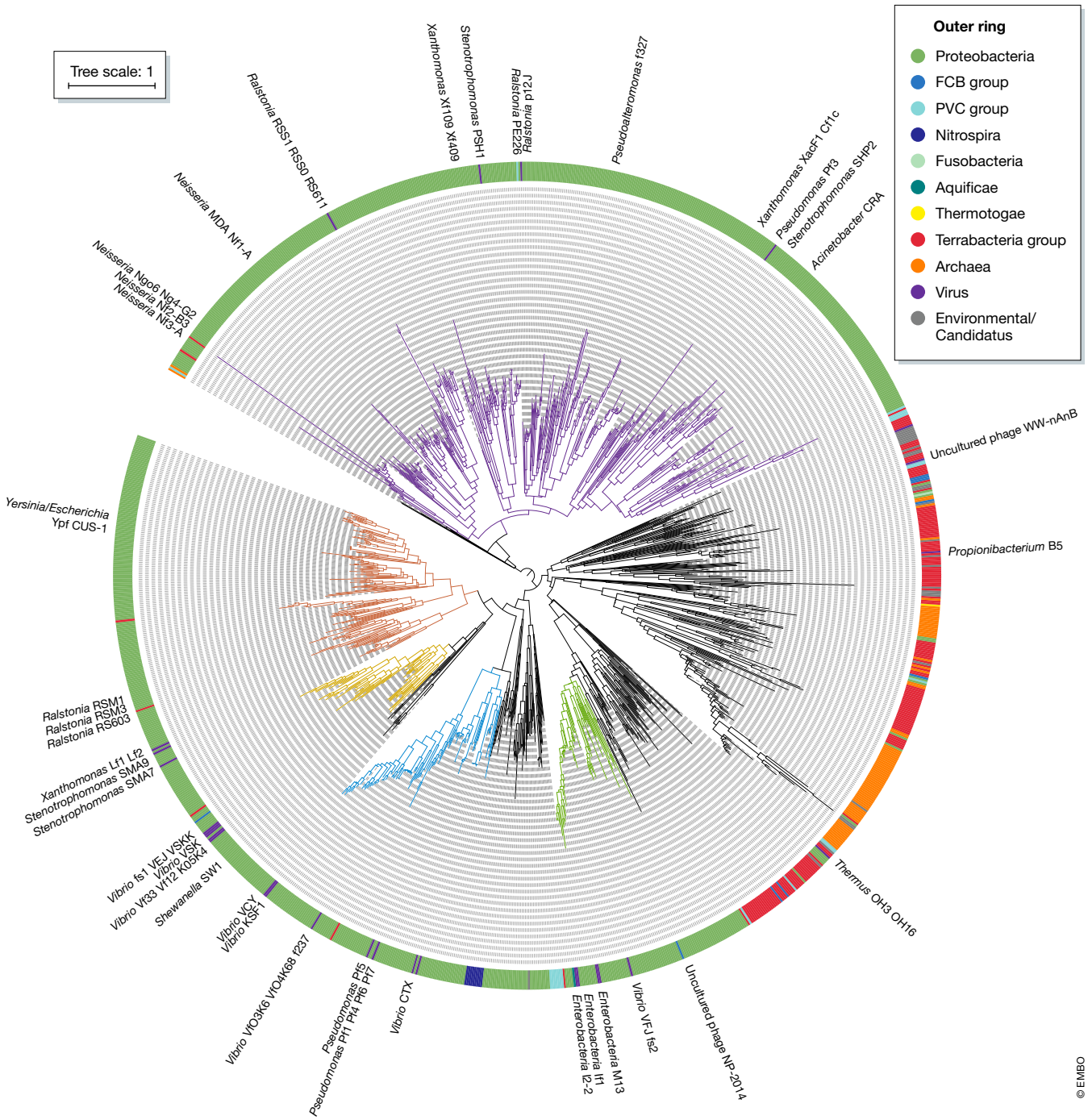

Figure 6. Ff discovery through gene signatures in prokaryote (host) chromosomes.

Zot domains are widely distributed throughout prokaryotic organisms including Gram-negative, Gram-positive and Archaeal organisms. Proteins from the UniRef90 database (representing 2,205 UniProtKB entries) with predicted "Zot" domains (PF05707) are represented in a phylogenetic tree. The taxonomic kingdom (or bacterial superphylum) is indicated in the outer ring. Branches/clades containing the known filamentous phages are coloured according to clades described in Fig 3. PF05707 domains were aligned and a tree was built with RAxML "PROTGAMMAAUTO" criteria (final model used = BLOSUM62) and "autoMRE_ING" bootstrap convergence test and midpoint rooted.

chromosome to form new phage genomes for amplification and virion assembly. This poses a problem when terminating rolling circle replication, as to reach the positive strand origin for termination the pII/DNA polymerase would have to replicate the entire bacterial chromosome. Consequently, the CTXφ prophage is always found integrated adjacent to a second *Vibrio* prophage, a satellite phage (a prophage lacking phage morphogenesis genes which uses a second phage to produce virions), or in a duplicated form, where

it utilizes the neighbouring prophage positive strand origin to terminate and release ssDNA phage genomes. A consequence of this is that the resulting phages contain fragments of the second prophage [183] (Fig 5).

The CTXφ prophage can be extracted under specific conditions—if CTXφ infects a strain already harbouring a reversibly integrated prophage such as VGJ. CTXφ can integrate adjacent to the VGJ prophage (using the *dif/att* site of the prophage) resulting in a VGJ-CTX module flanked by two intact functional *att* sites which can subsequently be used by the host Xer recombinase machinery to extract a hybrid VGJ-CTX genome that can undergo rolling circle replication and be packaged into functional virions containing the VGJ-CTX hybrid genome with the VGJ phage proteins [179,180,184,185]. This interplay of *Vibrio* prophage integration, chimera formation, excision and potential horizontal transfer results in the diverse concatenated prophage arrays observed at *dif* integration sites in *Vibrio* genomes [186,187].

There are three distinct examples of integration methods using filamentous phage-encoded recombinase, though there are limited mechanistic insights. *Pseudomonas* Pf4 and Pf5 phages are integrated into the chromosome at a site encoding a $tRNA^{Gly}$, whereas Pf6 is integrated at the $tRNA^{Met}$ site. These phages encode a tyrosine recombinase of the phage integrase family, protein which presumably facilitates this integration. The *Ralstonia* RSM phages integrate into the $tRNA^{Ser}$ site and encode their own serine recombinase of the resolvase/invertase family, which presumably facilitates this integration. Similar prophages are detected in the genomes of other species of Burkholderiaceae, such as the human pathogen *Burkholderia pseudomallei* [188]. The *Neisseria* prophages may have a more promiscuous integration mechanism, where they are integrated into a short repeat region called dRS3; in *N. meningitidis*, there are more than 250 of these sites present on the chromosome. The Nf typically encode a Piv/MooV family transposase, and it is thought that this mediates the insertion and excision (transposition) of the prophage genome via a method similar to IS110 transposons [171,173].

Prophages as a means to discover new Ff sequences (and new Ff applications)

As the pI N-terminal Zot domain (Pfam PF05707) seems to be characteristic of and unique to filamentous phages and prophages, this sequence signature can be used to gauge the prevalence of undocumented filamentous phages in genome and metagenome sequence data. There are approximately 2,300 proteins with PF05707 domains present in the UniProtKB (2018_09) database with almost all of them (> 99%) associated with prokaryotic genomes and likely from filamentous phage origins. There are representatives across a wide range of prokaryotes including various phyla from Gram-negative and Gram-positive bacteria, as well as Archaea. Thus, there are potentially thousands of uncharacterized filamentous prophages present within the prokaryotic genomic repertoire (Fig 6). Furthermore, searching the Joint Genome Institutes Integrated Microbial Genomes and Microbiomes database returns more than 300,000 genes from metagenome sequences predicted to contain the PF05707 domains.

Our appreciation of this diversity of filamentous phages heralds an exciting phase of further discovery, about their biology and their evolution (see also Box 1). A massive restructuring of the taxonomy of phages, undertaken by the International Committee on Taxonomy of Viruses (ICTV), put emphasis on genome sequence information [189–191]. This led to a complete restructuring of the Inoviridae family into seven genera, but left the bulk of the described filamentous phages as "unclassified" or "unassigned", again suggesting that we are only seeing a small cross section of the diversity of these useful and enigmatic viruses. The previous *Plectrovirus* genus containing the mollicute-infecting rod-shaped viruses has been divided into the genera *Plectrovirus* (containing the Acholeplasma virus L51) and the *Vespertiliovirus* (containing the Spiroplasma viruses). The previously defined genus *Inovirus* containing the "classic" filamentous phages has been divided into five new genera: *Inovirus, Habenivirus, Fibrovirus, Lineavirus* and *Saetivirus*; and many of the previously classified species have been shifted into an "unclassified" genus category. More recently, there has been a further push away from morphological taxonomy, to integrate more metagenomic data into viral taxonomy and develop a universal method to classify viruses [192–194]. Appling these methods to the Inoviridae family results in three unrelated groups representing the *Plectrovirus* genus, the *Vespertiliovirus* genus and the third diverse group comprising all the related classic filamentous phages described in this review, with *Saetivirus*, *Lineavirus* and *Inovirus* in one clade, acutely separated from a clade formed by *Fibrovirus*, *Habenivirus* and other currently unassigned groups [192].

Given how beneficial filamentous phages have been to our understanding of fundamental aspects of bacterial cell biology (e.g. membrane biogenesis, protein secretion, DNA recombination and replication) and developments in biotechnology, as well as their crucial roles in promoting bacterial virulence and shaping bacterial communities, this new age of filamentous phage discovery and characterization is one of promise and possibility.

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Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Edwards RA, Rohwer F (2005) Viral metagenomics. *Nat Rev Microbiol* 3: 504 – 510
2. Hatfull GF, Hendrix RW (2011) Bacteriophages and their genomes. *Curr Opin Virol* 1: 298 – 303
3. Thurber RV (2009) Current insights into phage biodiversity and biogeography. *Curr Opin Microbiol* 12: 582 – 587
4. Weinbauer MG (2004) Ecology of prokaryotic viruses. *FEMS Microbiol Rev* 28: 127 – 181
5. Casjens SR, Hendrix RW (2015) Bacteriophage lambda: early pioneer and still relevant. *Virology* 479–480: 310 – 330
6. Zhu B (2014) Bacteriophage T7 DNA polymerase - sequenase. *Front Microbiol* 5: 181
7. Wang W, Li Y, Wang Y, Shi C, Li C, Li Q, Linhardt RJ (2018) Bacteriophage T7 transcription system: an enabling tool in synthetic biology. *Biotechnol Adv* 36: 2129 – 2137

8. Sanger F, Coulson AR, Friedmann T, Air GM, Barrell BG, Brown NL, Fiddes JC, Hutchison CA III, Slocombe PM, Smith M (1978) The nucleotide sequence of bacteriophage phiX174. *J Mol Biol* 125: 225 – 246
9. Eisenberg S, Griffith J, Kornberg A (1977) phiX174 cistron A protein is a multifunctional enzyme in DNA replication. *Proc Natl Acad Sci USA* 74: 3198 – 3202
10. Smith HO, Hutchison CA III, Pfannkoch C, Venter JC (2003) Generating a synthetic genome by whole genome assembly: phiX174 bacteriophage from synthetic oligonucleotides. *Proc Natl Acad Sci USA* 100: 15440 – 15445
11. Pichon X, Lagha M, Mueller F, Bertrand E (2018) A growing toolbox to image gene expression in single cells: sensitive approaches for demanding challenges. *Mol Cell* 71: 468 – 480
12. Baba T, Bae T, Schneewind O, Takeuchi F, Hiramatsu K (2008) Genome sequence of *Staphylococcus aureus* strain Newman and comparative analysis of staphylococcal genomes: polymorphism and evolution of two major pathogenicity islands. *J Bacteriol* 190: 300 – 310
13. Bikard D, Euler CW, Jiang W, Nussenzweig PM, Goldberg GW, Duportet X, Fischetti VA, Marraffini LA (2014) Exploiting CRISPR-Cas nucleases to produce sequence-specific antimicrobials. *Nat Biotechnol* 32: 1146 – 1150
14. Hofschneider PH (1963) Untersuchungen uber kleine *E. coli* K 12 bakteriophagen 1 Und 2 mitteilung. *Z Naturforsch Pt B* 18: 203 – 210
15. Marvin DA, Hoffmann-Berling H (1963) Physical and chemical properties of two new small bacteriophages. *Nature* 197: 517
16. Zinder ND, Valentine RC, Roger M, Stoeckenius W (1963) F1, a rod-shaped male-specific bacteriophage that contains DNA. *Virology* 20: 638 – 640
17. Loeb T (1960) Isolation of a bacteriophage specific for the F plus and Hfr mating types of *Escherichia coli* K-12. *Science* 131: 932 – 933
18. Messing J (1993) M13 cloning vehicles. Their contribution to DNA sequencing. *Methods Mol Biol* 23: 9 – 22
19. Rakonjac J, Bennett NJ, Spagnuolo J, Gagic D, Russel M (2011) Filamentous bacteriophage: biology, phage display and nanotechnology applications. *Curr Issues Mol Biol* 13: 51 – 76
20. Smith GP (1985) Filamentous fusion phage: novel expression vectors that display cloned antigens on the virion surface. *Science* 228: 1315 – 1317
21. McCafferty J, Griffiths AD, Winter G, Chiswell DJ (1990) Phage antibodies: filamentous phage displaying antibody variable domains. *Nature* 348: 552 – 554
22. Parmley SF, Smith GP (1988) Antibody-selectable filamentous fd phage vectors: affinity purification of target genes. *Gene* 73: 305 – 318
23. Marvin DA, Symmons MF, Straus SK (2014) Structure and assembly of filamentous bacteriophages. *Prog Biophys Mol Biol* 114: 80 – 122
24. Day LA, Marzec CJ, Reisberg SA, Casadevall A (1988) DNA packing in filamentous bacteriophages. *Annu Rev Biophys Biophys Chem* 17: 509 – 539
25. Greenwood J, Hunter GJ, Perham RN (1991) Regulation of filamentous bacteriophage length by modification of electrostatic interactions between coat protein and DNA. *J Mol Biol* 217: 223 – 227
26. Symmons MF, Welsh LC, Nave C, Marvin DA, Perham RN (1995) Matching electrostatic charge between DNA and coat protein in filamentous bacteriophage. Fibre diffraction of charge-deletion mutants. *J Mol Biol* 245: 86 – 91
27. Liu DJ, Day LA (1994) Pf1 virus structure: helical coat protein and DNA with paraxial phosphates. *Science* 265: 671 – 674
28. Tsuboi M, Tsunoda M, Overman SA, Benevides JM, Thomas GJ Jr (2010) A structural model for the single-stranded DNA genome of filamentous bacteriophage Pf1. *Biochemistry* 49: 1737 – 1743
29. Grant RA, Lin TC, Konigsberg W, Webster RE (1981) Structure of the filamentous bacteriophage fl. Location of the A, C, and D minor coat proteins. *J Biol Chem* 256: 539 – 546
30. Lee SW, Mao C, Flynn CE, Belcher AM (2002) Ordering of quantum dots using genetically engineered viruses. *Science* 296: 892 – 895
31. Holliger P, Riechmann L, Williams RL (1999) Crystal structure of the two N-terminal domains of g3p from filamentous phage fd at 1.9 A: evidence for conformational lability. *J Mol Biol* 288: 649 – 657
32. Rakonjac J, Feng J, Model P (1999) Filamentous phage are released from the bacterial membrane by a two-step mechanism involving a short C-terminal fragment of pIII. *J Mol Biol* 289: 1253 – 1265
33. Endemann H, Model P (1995) Location of filamentous phage minor coat proteins in phage and in infected cells. *J Mol Biol* 250: 496 – 506
34. Bennett NJ, Gagic D, Sutherland-Smith AJ, Rakonjac J (2011) Characterization of a dual-function domain that mediates membrane insertion and excision of Ff filamentous bacteriophage. *J Mol Biol* 411: 972 – 985
35. Russel M, Model P (1989) Genetic analysis of the filamentous bacteriophage packaging signal and of the proteins that interact with it. *J Virol* 63: 3284 – 3295
36. Faruque SM, Mekalanos JJ (2012) Phage-bacterial interactions in the evolution of toxigenic *Vibrio cholerae*. *Virulence* 3: 556 – 565
37. Hassan F, Kamruzzaman M, Mekalanos JJ, Faruque SM (2010) Satellite phage TLCphi enables toxigenic conversion by CTX phage through dif site alteration. *Nature* 467: 982 – 985
38. Karaolis DK, Somara S, Maneval DR Jr, Johnson JA, Kaper JB (1999) A bacteriophage encoding a pathogenicity island, a type-IV pilus and a phage receptor in cholera bacteria. *Nature* 399: 375 – 379
39. Faruque SM, Zhu J, Asadulghani Kamruzzaman M, Mekalanos JJ (2003) Examination of diverse toxin-coregulated pilus-positive *Vibrio cholerae* strains fails to demonstrate evidence for Vibrio pathogenicity island phage. *Infect Immun* 71: 2993 – 2999
40. Murphy RA, Boyd EF (2008) Three pathogenicity islands of *Vibrio cholerae* can excise from the chromosome and form circular intermediates. *J Bacteriol* 190: 636 – 647
41. O'Shea YA, Boyd EF (2002) Mobilization of the *Vibrio* pathogenicity island between *Vibrio cholerae* isolates mediated by CP-T1 generalized transduction. *FEMS Microbiol Lett* 214: 153 – 157
42. Rajanna C, Wang J, Zhang D, Xu Z, Ali A, Hou YM, Karaolis DK (2003) The vibrio pathogenicity island of epidemic *Vibrio cholerae* forms precise extrachromosomal circular excision products. *J Bacteriol* 185: 6893 – 6901
43. Castillo D, Kauffman K, Hussain F, Kalatzis P, Rorbo N, Polz MF, Middelboe M (2018) Widespread distribution of prophage-encoded virulence factors in marine *Vibrio* communities. *Sci Rep* 8: 9973
44. Xue H, Xu Y, Boucher Y, Polz MF (2012) High frequency of a novel filamentous phage, VCY phi, within an environmental *Vibrio cholerae* population. *Appl Environ Microbiol* 78: 28 – 33
45. Weynberg KD, Voolstra CR, Neave MJ, Buerger P, van Oppen MJ (2015) From cholera to corals: viruses as drivers of virulence in a major coral bacterial pathogen. *Sci Rep* 5: 17889
46. Sakib SN, Reddi G, Almagro-Moreno S (2018) Environmental role of pathogenic traits in *Vibrio cholerae*. *J Bacteriol* 200: e00795-17
47. Renda BA, Chan C, Parent KN, Barrick JE (2016) Emergence of a competence-reducing filamentous phage from the genome of acinetobacter baylyi ADP1. *J Bacteriol* 198: 3209 – 3219
48. Knezevic P, Voet M, Lavigne R (2015) Prevalence of Pf1-like (pro)phage genetic elements among *Pseudomonas aeruginosa* isolates. *Virology* 483: 64 – 71

49. Mai-Prochnow A, Hui JG, Kjelleberg S, Rakonjac J, McDougald D, Rice SA (2015) Big things in small packages: the genetics of filamentous phage and effects on fitness of their host. *FEMS Microbiol Rev* 39: 465 – 487
50. Whiteley M, Bangera MG, Bumgarner RE, Parsek MR, Teitzel GM, Lory S, Greenberg EP (2001) Gene expression in *Pseudomonas aeruginosa* biofilms. *Nature* 413: 860 – 864
51. Platt MD, Schurr MJ, Sauer K, Vazquez G, Kukavica-Ibrulj I, Potvin E, Levesque RC, Fedynak A, Brinkman FS, Schurr J *et al* (2008) Proteomic, microarray, and signature-tagged mutagenesis analyses of anaerobic *Pseudomonas aeruginosa* at pH 6.5, likely representing chronic, late-stage cystic fibrosis airway conditions. *J Bacteriol* 190: 2739 – 2758
52. Secor PR, Sweere JM, Michaels LA, Malkovskiy AV, Lazzareschi D, Katznelson E, Rajadas J, Birnbaum ME, Arrigoni A, Braun KR *et al* (2015) Filamentous bacteriophage promote biofilm assembly and function. *Cell Host Microbe* 18: 549 – 559
53. Rice SA, Tan CH, Mikkelsen PJ, Kung V, Woo J, Tay M, Hauser A, McDougald D, Webb JS, Kjelleberg S (2009) The biofilm life cycle and virulence of *Pseudomonas aeruginosa* are dependent on a filamentous prophage. *ISME J* 3: 271 – 282
54. Webb JS, Thompson LS, James S, Charlton T, Tolker-Nielsen T, Koch B, Givskov M, Kjelleberg S (2003) Cell death in *Pseudomonas aeruginosa* biofilm development. *J Bacteriol* 185: 4585 – 4592
55. Webb JS, Lau M, Kjelleberg S (2004) Bacteriophage and phenotypic variation in *Pseudomonas aeruginosa* biofilm development. *J Bacteriol* 186: 8066 – 8073
56. Secor PR, Sass G, Nazik H, Stevens DA (2017) Effect of acute predation with bacteriophage on intermicrobial aggression by *Pseudomonas aeruginosa*. *PLoS ONE* 12: e0179659
57. Kuo TT, Huang TC, Chow TY (1969) A filamentous bacteriophage from *Xanthomonas oryzae*. *Virology* 39: 548 – 555
58. Kuo TT, Lin YH, Huang CM, Chang SF, Dai H, Feng TY (1987) The lysogenic cycle of the filamentous phage CfIt from *Xanthomonas campestris* pv. citri. *Virology* 156: 305 – 312
59. Kuo TT, Chao YS, Lin YH, Lin BY, Liu LF, Feng TY (1987) Integration of the DNA of filamentous bacteriophage CfIt into the chromosomal DNA of its host. *J Virol* 61: 60 – 65
60. Kuo TT, Chiang CC, Chen SY, Lin JH, Kuo JL (1994) A long lytic cycle in filamentous phage Cf1tv infecting *Xanthomonas campestris* pv. citri. *Arch Virol* 135: 253 – 264
61. Yeh TY (2017) Complete nucleotide sequence of a new filamentous phage, Xf109, which integrates its genome into the chromosomal DNA of *Xanthomonas oryzae*. *Arch Virol* 162: 567 – 572
62. Askora A, Yamada T (2015) Two different evolutionary lines of filamentous phages in *Ralstonia solanacearum*: their effects on bacterial virulence. *Front Genet* 6: 217
63. Yamada T (2013) Filamentous phages of *Ralstonia solanacearum*: double-edged swords for pathogenic bacteria. *Front Microbiol* 4: 325
64. Addy HS, Askora A, Kawasaki T, Fujie M, Yamada T (2012) Utilization of filamentous phage phi RSM3 to control bacterial wilt caused by *Ralstonia solanacearum*. *Plant Dis* 96: 1204 – 1209
65. Addy HS, Askora A, Kawasaki T, Fujie M, Yamada T (2012) Loss of virulence of the phytopathogen *Ralstonia solanacearum* through infection by phiRSM filamentous phages. *Phytopathology* 102: 469 – 477
66. Ahmad AA, Stulberg MJ, Huang Q (2017) Prophage Rs551 and its repressor gene orf14 reduce virulence and increase competitive fitness of its *Ralstonia solanacearum* carrier strain UW551. *Front Microbiol* 8: 2480
67. Ahmad AA, Stulberg MJ, Mershon JP, Mollov DS, Huang Q (2017) Molecular and biological characterization of varphiRs551, a filamentous bacteriophage isolated from a race 3 biovar 2 strain of *Ralstonia solanacearum*. *PLoS ONE* 12: e0185034
68. Askora A, Kawasaki T, Usami S, Fujie M, Yamada T (2009) Host recognition and integration of filamentous phage phiRSM in the phytopathogen, *Ralstonia solanacearum*. *Virology* 384: 69 – 76
69. Kawasaki T, Nagata S, Fujiwara A, Satsuma H, Fujie M, Usami S, Yamada T (2007) Genomic characterization of the filamentous integrative bacteriophages {phi}RSS1 and {phi}RSM1, which infect *Ralstonia solanacearum*. *J Bacteriol* 189: 5792 – 5802
70. Narulita E, Addy HS, Kawasaki T, Fujie M, Yamada T (2016) The involvement of the PilQ secretin of type IV pili in phage infection in *Ralstonia solanacearum*. *Biochem Biophys Res Commun* 469: 868 – 872
71. Addy HS, Askora A, Kawasaki T, Fujie M, Yamada T (2012) The filamentous phage varphiRSS1 enhances virulence of phytopathogenic *Ralstonia solanacearum* on tomato. *Phytopathology* 102: 244 – 251
72. Van TT, Yoshida S, Miki K, Kondo A, Kamei K (2014) Genomic characterization of varphiRS603, a filamentous bacteriophage that is infectious to the phytopathogen *Ralstonia solanacearum*. *Microbiol Immunol* 58: 697 – 700
73. Van TT, Yoshida S, Miki K, Kondo A, Kamei K (2015) Complete genome sequence of a filamentous bacteriophage, RS611, that infects the phytopathogen *Ralstonia solanacearum*. *Arch Virol* 160: 865 – 867
74. Chan B, Miyamoto H, Taniguchi H, Yoshida S (2002) Isolation and genetic characterization of a novel filamentous bacteriophage, a deleted form of phage f237, from a pandemic *Vibrio* parahaemolyticus O4:K68 strain. *Microbiol Immunol* 46: 565 – 569
75. Jian H, Xiong L, Xu G, Xiao X (2016) Filamentous phage SW1 is active and influences the transcriptome of the host at high-pressure and low-temperature. *Environ Microbiol Rep* 8: 358 – 362
76. Jian H, Xu J, Xiao X, Wang F (2012) Dynamic modulation of DNA replication and gene transcription in deep-sea filamentous phage SW1 in response to changes of host growth and temperature. *PLoS ONE* 7: e41578
77. Wang F, Wang F, Li Q, Xiao X (2007) A novel filamentous phage from the deep-sea bacterium *Shewanella piezotolerans* WP3 is induced at low temperature. *J Bacteriol* 189: 7151 – 7153
78. Yang XW, Jian HH, Wang FP (2015) pSW2, a novel low-temperature-inducible gene expression vector based on a filamentous phage of the deep-sea bacterium *Shewanella piezotolerans* WP3. *Appl Environ Microbiol* 81: 5519 – 5526
79. Nagayoshi Y, Kumagae K, Mori K, Tashiro K, Nakamura A, Fujino Y, Hiromasa Y, Iwamoto T, Kuhara S, Ohshima T *et al* (2016) Physiological properties and genome structure of the hyperthermophilic filamentous phage phiOH3 which infects *Thermus thermophilus* HB8. *Front Microbiol* 7: 50
80. Fasano A, Baudry B, Pumplin DW, Wasserman SS, Tall BD, Ketley JM, Kaper JB (1991) Vibrio cholerae produces a second enterotoxin, which affects intestinal tight junctions. *Proc Natl Acad Sci USA* 88: 5242 – 5246
81. Perez-Reytor D, Jana V, Pavez L, Navarrete P, Garcia K (2018) Accessory toxins of Vibrio pathogens and their role in epithelial disruption during infection. *Front Microbiol* 9: 2248
82. Baudry B, Fasano A, Ketley J, Kaper JB (1992) Cloning of a gene (zot) encoding a new toxin produced by *Vibrio cholerae*. *Infect Immun* 60: 428 – 434

83. Di Pierro M, Lu R, Uzzau S, Wang W, Margaretten K, Pazzani C, Maimone F, Fasano A (2001) Zonula occludens toxin structure-function analysis. Identification of the fragment biologically active on tight junctions and of the zonulin receptor binding domain. *J Biol Chem* 276: 19160 – 19165
84. Goldblum SE, Rai U, Tripathi A, Thakar M, De Leo L, Di Toro N, Not T, Ramachandran R, Puche AC, Hollenberg MD *et al* (2011) The active Zot domain (aa 288-293) increases ZO-1 and myosin 1C serine/threonine phosphorylation, alters interaction between ZO-1 and its binding partners, and induces tight junction disassembly through proteinase activated receptor 2 activation. *FASEB J* 25: 144 – 158
85. Uzzau S, Cappuccinelli P, Fasano A (1999) Expression of Vibrio cholerae zonula occludens toxin and analysis of its subcellular localization. *Microb Pathog* 27: 377 – 385
86. Schmidt E, Kelly SM, van der Walle CF (2007) Tight junction modulation and biochemical characterisation of the zonula occludens toxin C- and N-termini. *FEBS Lett* 581: 2974 – 2980
87. Murugaiyan S, Bae JY, Wu J, Lee SD, Um HY, Choi HK, Chung E, Lee JH, Lee SW (2011) Characterization of filamentous bacteriophage PE226 infecting *Ralstonia solanacearum* strains. *J Appl Microbiol* 110: 296 – 303
88. Hagemann M, Hasse D, Berg G (2006) Detection of a phage genome carrying a zonula occludens like toxin gene (zot) in clinical isolates of *Stenotrophomonas maltophilia*. *Arch Microbiol* 185: 449 – 458
89. Campos J, Martinez E, Suzarte E, Rodriguez BL, Marrero K, Silva Y, Ledon T, del Sol R, Fando R (2003) VGJ phi, a novel filamentous phage of *Vibrio cholerae*, integrates into the same chromosomal site as CTX phi. *J Bacteriol* 185: 5685 – 5696
90. Deng LW, Malik P, Perham RN (1999) Interaction of the globular domains of pIII protein of filamentous bacteriophage fd with the F-pilus of *Escherichia coli*. *Virology* 253: 271 – 277
91. Holland SJ, Sanz C, Perham RN (2006) Identification and specificity of pilus adsorption proteins of filamentous bacteriophages infecting *Pseudomonas aeruginosa*. *Virology* 345: 540 – 548
92. Jouravleva EA, McDonald GA, Marsh JW, Taylor RK, Boesman-Finkelstein M, Finkelstein RA (1998) The *Vibrio cholerae* mannose-sensitive hemagglutinin is the receptor for a filamentous bacteriophage from *V. cholerae* O139. *Infect Immun* 66: 2535 – 2539
93. Yang YC, Chou CP, Kuo TT, Lin SH, Yang MK (2004) PilR enhances the sensitivity of *Xanthomonas axonopodis* pv. citri to the infection of filamentous bacteriophage Cf. *Curr Microbiol* 48: 251 – 261
94. Jacobson A (1972) Role of F pili in the penetration of bacteriophage fl. *J Virol* 10: 835 – 843
95. Gutierrez MA (2017) The minor pilin TcpB is located at the tip of the toxin co-regulated pilus of *Vibrio cholerae* and is the receptor for the filamentous phage CTXφ. In Department of Molecular Biology and Biochemistry, Faculty of Science Simon Fraser University
96. Clarke M, Maddera L, Harris RL, Silverman PM (2008) F-pili dynamics by live-cell imaging. *Proc Natl Acad Sci USA* 105: 17978 – 17981
97. Godlewska R, Wisniewska K, Pietras Z, Jagusztyn-Krynicka EK (2009) Peptidoglycan-associated lipoprotein (Pal) of Gram-negative bacteria: function, structure, role in pathogenesis and potential application in immunoprophylaxis. *FEMS Microbiol Lett* 298: 1 – 11
98. Click EM, Webster RE (1997) Filamentous phage infection: required interactions with the TolA protein. *J Bacteriol* 179: 6464 – 6471
99. Heilpern AJ, Waldor MK (2000) CTXphi infection of *Vibrio cholerae* requires the tolQRA gene products. *J Bacteriol* 182: 1739 – 1747
100. Riechmann L, Holliger P (1997) The C-terminal domain of TolA is the coreceptor for filamentous phage infection of *E. coli*. *Cell* 90: 351 – 360
101. Holliger P, Riechmann L (1997) A conserved infection pathway for filamentous bacteriophages is suggested by the structure of the membrane penetration domain of the minor coat protein g3p from phage fd. *Structure* 5: 265 – 275
102. Boeke JD, Model P, Zinder ND (1982) Effects of bacteriophage f1 gene III protein on the host cell membrane. *Mol Gen Genet* 186: 185 – 192
103. Glaser-Wuttke G, Keppner J, Rasched I (1989) Pore-forming properties of the adsorption protein of filamentous phage fd. *Biochim Biophys Acta* 985: 239 – 247
104. Click EM, Webster RE (1998) The TolQRA proteins are required for membrane insertion of the major capsid protein of the filamentous phage f1 during infection. *J Bacteriol* 180: 1723 – 1728
105. Smilowitz H (1974) Bacteriophage f1 infection: fate of the parental major coat protein. *J Virol* 13: 94 – 99
106. Armstrong J, Hewitt JA, Perham RN (1983) Chemical modification of the coat protein in bacteriophage fd and orientation of the virion during assembly and disassembly. *EMBO J* 2: 1641 – 1646
107. Baas PD (1985) DNA replication of single-stranded *Escherichia coli* DNA phages. *Biochim Biophys Acta* 825: 111 – 139
108. Ruiz-Maso JA, Macho NC, Bordanaba-Ruiseco L, Espinosa M, Coll M, Del Solar G (2015) Plasmid rolling-circle replication. *Microbiol Spectr* 3: PLAS-0035-2014
109. Higashitani A, Higashitani N, Horiuchi K (1997) Minus-strand origin of filamentous phage versus transcriptional promoters in recognition of RNA polymerase. *Proc Natl Acad Sci USA* 94: 2909 – 2914
110. Higashitani N, Higashitani A, Guan ZW, Horiuchi K (1996) Recognition mechanisms of the minus-strand origin of phage f1 by *Escherichia coli* RNA polymerase. *Genes Cells* 1: 829 – 841
111. Zenkin N, Naryshkina T, Kuznedelov K, Severinov K (2006) The mechanism of DNA replication primer synthesis by RNA polymerase. *Nature* 439: 617 – 620
112. Zenkin N, Severinov K (2004) The role of RNA polymerase sigma subunit in promoter-independent initiation of transcription. *Proc Natl Acad Sci USA* 101: 4396 – 4400
113. Kim MH, Hines JC, Ray DS (1981) Viable deletions of the M13 complementary strand origin. *Proc Natl Acad Sci USA* 78: 6784 – 6788
114. Meyer TF, Geider K (1979) Bacteriophage fd gene II-protein. II. Specific cleavage and relaxation of supercoiled RF from filamentous phages. *J Biol Chem* 254: 12642 – 12646
115. Horiuchi K (1997) Initiation mechanisms in replication of filamentous phage DNA. *Genes Cells* 2: 425 – 432
116. Asano S, Higashitani A, Horiuchi K (1999) Filamentous phage replication initiator protein gpII forms a covalent complex with the 5′ end of the nick it introduced. *Nucleic Acids Res* 27: 1882 – 1889
117. Meyer TF, Geider K (1982) Enzymatic synthesis of bacteriophage fd viral DNA. *Nature* 296: 828 – 832
118. Martinez E, Campos-Gomez J (2016) Pf filamentous phage requires UvrD for replication in *Pseudomonas aeruginosa*. *mSphere* 1: e00104 – e00115
119. Martinez E, Campos-Gomez J, Barre FX (2016) CTXvarphi: exploring new alternatives in host factor-mediated filamentous phage replications. *Bacteriophage* 6: e1128512
120. Martinez E, Paly E, Barre FX (2015) CTXphi replication depends on the histone-like HU protein and the UvrD helicase. *PLoS Genet* 11: e1005256
121. Lerner TJ, Model P (1981) The "steady state" of coliphage f1: DNA synthesis late in infection. *Virology* 115: 282 – 294

122. Guan Y, Zhang H, Wang AH (1995) Electrostatic potential distribution of the gene V protein from Ff phage facilitates cooperative DNA binding: a model of the GVP-ssDNA complex. *Protein Sci* 4: 187 – 197
123. Alberts B, Frey L, Delius H (1972) Isolation and characterization of gene 5 protein of filamentous bacterial viruses. *J Mol Biol* 68: 139 – 152
124. Fulford W, Model P (1988) Bacteriophage f1 DNA replication genes. II. The roles of gene V protein and gene II protein in complementary strand synthesis. *J Mol Biol* 203: 39 – 48
125. Michel B, Zinder ND (1989) Translational repression in bacteriophage f1: characterization of the gene V protein target on the gene II mRNA. *Proc Natl Acad Sci USA* 86: 4002 – 4006
126. Zaman GJ, Kaan AM, Schoenmakers JG, Konings RN (1992) Gene V protein-mediated translational regulation of the synthesis of gene II protein of the filamentous bacteriophage M13: a dispensable function of the filamentous-phage genome. *J Bacteriol* 174: 595 – 600
127. Fulford W, Model P (1988) Regulation of bacteriophage f1 DNA replication. I. New functions for genes II and X. *J Mol Biol* 203: 49 – 62
128. Kokoska RJ, Steege DA (1998) Appropriate expression of filamentous phage f1 DNA replication genes II and X requires RNase E-dependent processing and separate mRNAs. *J Bacteriol* 180: 3245 – 3249
129. Kutter E, Bryan D, Ray G, Brewster E, Blasdel B, Guttman B (2018) From host to phage metabolism: hot tales of phage T4's takeover of *E. coli*. *Viruses* 10: E387
130. Rapoza MP, Webster RE (1993) The filamentous bacteriophage assembly proteins require the bacterial SecA protein for correct localization to the membrane. *J Bacteriol* 175: 1856 – 1859
131. Haigh NG, Webster RE (1999) The pI and pXI assembly proteins serve separate and essential roles in filamentous phage assembly. *J Mol Biol* 293: 1017 – 1027
132. Rapoza MP, Webster RE (1995) The products of gene I and the overlapping in-frame gene XI are required for filamentous phage assembly. *J Mol Biol* 248: 627 – 638
133. Feng JN, Russel M, Model P (1997) A permeabilized cell system that assembles filamentous bacteriophage. *Proc Natl Acad Sci USA* 94: 4068 – 4073
134. Feng JN, Model P, Russel M (1999) A trans-envelope protein complex needed for filamentous phage assembly and export. *Mol Microbiol* 34: 745 – 755
135. Dunstan RA, Hay ID, Wilksch JJ, Schittenhelm RB, Purcell AW, Clark J, Costin A, Ramm G, Strugnell RA, Lithgow T (2015) Assembly of the secretion pores GspD, Wza and CsgG into bacterial outer membranes does not require the Omp85 proteins BamA or TamA. *Mol Microbiol* 97: 616 – 629
136. Opalka N, Beckmann R, Boisset N, Simon MN, Russel M, Darst SA (2003) Structure of the filamentous phage pIV multimer by cryo-electron microscopy. *J Mol Biol* 325: 461 – 470
137. Hay ID, Belousoff MJ, Dunstan RA, Bamert RS, Lithgow T (2018) Structure and membrane topography of the vibrio-type secretin complex from the type 2 secretion system of enteropathogenic *Escherichia coli*. *J Bacteriol* 200: e00521 – 17
138. Hay ID, Belousoff MJ, Lithgow T (2017) Structural basis of type 2 secretion system engagement between the inner and outer bacterial membranes. *MBio* 8: e01344 – 17
139. Yan Z, Yin M, Xu D, Zhu Y, Li X (2017) Structural insights into the secretin translocation channel in the type II secretion system. *Nat Struct Mol Biol* 24: 177 – 183
140. Spagnuolo J, Opalka N, Wen WX, Gagic D, Chabaud E, Bellini P, Bennett MD, Norris GE, Darst SA, Russel M *et al* (2010) Identification of the gate regions in the primary structure of the secretin pIV. *Mol Microbiol* 76: 133 – 150
141. Hu J, Worrall LJ, Hong C, Vuckovic M, Atkinson CE, Caveney N, Yu Z, Strynadka NCJ (2018) Cryo-EM analysis of the T3S injectisome reveals the structure of the needle and open secretin. *Nat Commun* 9: 3840
142. Davis BM, Lawson EH, Sandkvist M, Ali A, Sozhamannan S, Waldor MK (2000) Convergence of the secretory pathways for cholera toxin and the filamentous phage, CTXphi. *Science* 288: 333 – 335
143. Davis BM, Waldor MK (2003) Filamentous phages linked to virulence of *Vibrio cholerae*. *Curr Opin Microbiol* 6: 35 – 42
144. Bille E, Zahar JR, Perrin A, Morelle S, Kriz P, Jolley KA, Maiden MC, Dervin C, Nassif X, Tinsley CR (2005) A chromosomally integrated bacteriophage in invasive meningococci. *J Exp Med* 201: 1905 – 1913
145. Daefler S, Russel M, Model P (1997) Module swaps between related translocator proteins pIV(f1), pIV(IKe) and PulD: identification of a specificity domain. *J Mol Biol* 266: 978 – 992
146. de Groot A, Koster M, Gerard-Vincent M, Gerritse G, Lazdunski A, Tommassen J, Filloux A (2001) Exchange of Xcp (Gsp) secretion machineries between *Pseudomonas aeruginosa* and *Pseudomonas alcaligenes*: species specificity unrelated to substrate recognition. *J Bacteriol* 183: 959 – 967
147. Russel M (1993) Protein-protein interactions during filamentous phage assembly. *J Mol Biol* 231: 689 – 697
148. Rohrer J, Kuhn A (1990) The function of a leader peptide in translocating charged amino acyl residues across a membrane. *Science* 250: 1418 – 1421
149. Cao G, Dalbey RE (1994) Translocation of N-terminal tails across the plasma membrane. *EMBO J* 13: 4662 – 4669
150. Kiefer D, Hu X, Dalbey R, Kuhn A (1997) Negatively charged amino acid residues play an active role in orienting the Sec-independent Pf3 coat protein in the *Escherichia coli* inner membrane. *EMBO J* 16: 2197 – 2204
151. Kuhn A (1995) Major coat proteins of bacteriophage Pf3 and M13 as model systems for Sec-independent protein transport. *FEMS Microbiol Rev* 17: 185 – 190
152. Wickner W (1988) Mechanisms of membrane assembly: general lessons from the study of M13 coat protein and *Escherichia coli* leader peptidase. *Biochemistry* 27: 1081 – 1086
153. Xie K, Dalbey RE (2008) Inserting proteins into the bacterial cytoplasmic membrane using the Sec and YidC translocases. *Nat Rev Microbiol* 6: 234 – 244
154. de Gier JW, Scotti PA, Saaf A, Valent QA, Kuhn A, Luirink J, von Heijne G (1998) Differential use of the signal recognition particle translocase targeting pathway for inner membrane protein assembly in *Escherichia coli*. *Proc Natl Acad Sci USA* 95: 14646 – 14651
155. Grant RA, Lin TC, Webster RE, Konigsberg W (1981) Structure of filamentous bacteriophage: isolation, characterization, and localization of the minor coat proteins and orientation of the DNA. *Prog Clin Biol Res* 64: 413 – 428
156. Lopez J, Webster RE (1985) Assembly site of bacteriophage f1 corresponds to adhesion zones between the inner and outer membranes of the host cell. *J Bacteriol* 163: 1270 – 1274
157. Simons GF, Konings RN, Schoenmakers JG (1981) Genes VI, VII, and IX of phage M13 code for minor capsid proteins of the virion. *Proc Natl Acad Sci USA* 78: 4194 – 4198
158. Samuelson JC, Chen M, Jiang F, Moller I, Wiedmann M, Kuhn A, Phillips GJ, Dalbey RE (2000) YidC mediates membrane protein insertion in bacteria. *Nature* 406: 637 – 641

159. Samuelson JC, Jiang F, Yi L, Chen M, de Gier JW, Kuhn A, Dalbey RE (2001) Function of YidC for the insertion of M13 procoat protein in *Escherichia coli*: translocation of mutants that show differences in their membrane potential dependence and Sec requirement. *J Biol Chem* 276: 34847 – 34852
160. Serek J, Bauer-Manz G, Struhalla G, van den Berg L, Kiefer D, Dalbey R, Kuhn A (2004) *Escherichia coli* YidC is a membrane insertase for Sec-independent proteins. *EMBO J* 23: 294 – 301
161. Klenner C, Yuan J, Dalbey RE, Kuhn A (2008) The Pf3 coat protein contacts TM1 and TM3 of YidC during membrane biogenesis. *FEBS Lett* 582: 3967 – 3972
162. Cao G, Kuhn A, Dalbey RE (1995) The translocation of negatively charged residues across the membrane is driven by the electrochemical potential: evidence for an electrophoresis-like membrane transfer mechanism. *EMBO J* 14: 866 – 875
163. Haigh NG, Webster RE (1998) The major coat protein of filamentous bacteriophage f1 specifically pairs in the bacterial cytoplasmic membrane. *J Mol Biol* 279: 19 – 29
164. Nagler C, Nagler G, Kuhn A (2007) Cysteine residues in the transmembrane regions of M13 procoat protein suggest that oligomeric coat proteins assemble onto phage progeny. *J Bacteriol* 189: 2897 – 2905
165. Straus SK, Bo HE (2018) Filamentous bacteriophage proteins and assembly. *Subcell Biochem* 88: 261 – 279
166. Gailus V, Rasched I (1994) The adsorption protein of bacteriophage fd and its neighbour minor coat protein build a structural entity. *Eur J Biochem* 222: 927 – 931
167. Boeke JD, Model P (1982) A prokaryotic membrane anchor sequence: carboxyl terminus of bacteriophage f1 gene III protein retains it in the membrane. *Proc Natl Acad Sci USA* 79: 5200 – 5204
168. Gonzalez MD, Lichtensteiger CA, Caughlan R, Vimr ER (2002) Conserved filamentous prophage in *Escherichia coli* O18:K1:H7 and *Yersinia pestis* biovar orientalis. *J Bacteriol* 184: 6050 – 6055
169. Gonzalez MD, Lichtensteiger CA, Vimr ER (2001) Adaptation of signature-tagged mutagenesis to *Escherichia coli* K1 and the infant-rat model of invasive disease. *FEMS Microbiol Lett* 198: 125 – 128
170. Derbise A, Chenal-Francisque V, Pouillot F, Fayolle C, Prevost MC, Medigue C, Hinnebusch BJ, Carniel E (2007) A horizontally acquired filamentous phage contributes to the pathogenicity of the plague bacillus. *Mol Microbiol* 63: 1145 – 1157
171. Kawai M, Uchiyama I, Kobayashi I (2005) Genome comparison *in silico* in *Neisseria* suggests integration of filamentous bacteriophages by their own transposase. *DNA Res* 12: 389 – 401
172. Piekarowicz A, Majchrzak M, Klyz A, Adamczyk-Poplawska M (2006) Analysis of the filamentous bacteriophage genomes integrated into *Neisseria gonorrhoeae* FA1090 chromosome. *Pol J Microbiol* 55: 251 – 260
173. Skaar EP, Lecuyer B, Lenich AG, Lazio MP, Perkins-Balding D, Seifert HS, Karls AC (2005) Analysis of the Piv recombinase-related gene family of *Neisseria gonorrhoeae*. *J Bacteriol* 187: 1276 – 1286
174. Gibbs CP, Meyer TF (1996) Genome plasticity in *Neisseria gonorrhoeae*. *FEMS Microbiol Lett* 145: 173 – 179
175. Spencer-Smith R, Varkey EM, Fielder MD, Snyder LA (2012) Sequence features contributing to chromosomal rearrangements in *Neisseria gonorrhoeae*. *PLoS ONE* 7: e46023
176. Bille E, Ure R, Gray SJ, Kaczmarski EB, McCarthy ND, Nassif X, Maiden MC, Tinsley CR (2008) Association of a bacteriophage with meningococcal disease in young adults. *PLoS ONE* 3: e3885
177. Bille E, Meyer J, Jamet A, Euphrasie D, Barnier JP, Brissac T, Larsen A, Pelissier P, Nassif X (2017) A virulence-associated filamentous bacteriophage of *Neisseria meningitidis* increases host-cell colonisation. *PLoS Pathog* 13: e1006495
178. Castillo F, Benmohamed A, Szatmari G (2017) Xer site specific recombination: double and single recombinase systems. *Front Microbiol* 8: 453
179. Das B (2014) Mechanistic insights into filamentous phage integration in *Vibrio cholerae*. *Front Microbiol* 5: 650
180. Das B, Bischerour J, Barre FX (2011) VGJphi integration and excision mechanisms contribute to the genetic diversity of *Vibrio cholerae* epidemic strains. *Proc Natl Acad Sci USA* 108: 2516 – 2521
181. Das B, Bischerour J, Val ME, Barre FX (2010) Molecular keys of the tropism of integration of the cholera toxin phage. *Proc Natl Acad Sci USA* 107: 4377 – 4382
182. Val ME, Bouvier M, Campos J, Sherratt D, Cornet F, Mazel D, Barre FX (2005) The single-stranded genome of phage CTX is the form used for integration into the genome of *Vibrio cholerae*. *Mol Cell* 19: 559 – 566
183. McLeod SM, Kimsey HH, Davis BM, Waldor MK (2005) CTXphi and *Vibrio cholerae*: exploring a newly recognized type of phage-host cell relationship. *Mol Microbiol* 57: 347 – 356
184. Campos J, Martinez E, Izquierdo Y, Fando R (2010) VEJ{phi}, a novel filamentous phage of *Vibrio cholerae* able to transduce the cholera toxin genes. *Microbiology* 156: 108 – 115
185. Campos J, Martinez E, Marrero K, Silva Y, Rodriguez BL, Suzarte E, Ledon T, Fando R (2003) Novel type of specialized transduction for CTX phi or its satellite phage RS1 mediated by filamentous phage VGJ phi in *Vibrio cholerae*. *J Bacteriol* 185: 7231 – 7240
186. Chun J, Grim CJ, Hasan NA, Lee JH, Choi SY, Haley BJ, Taviani E, Jeon YS, Kim DW, Lee JH *et al* (2009) Comparative genomics reveals mechanism for short-term and long-term clonal transitions in pandemic *Vibrio cholerae*. *Proc Natl Acad Sci USA* 106: 15442 – 15447
187. Kim EJ, Lee CH, Nair GB, Kim DW (2015) Whole-genome sequence comparisons reveal the evolution of *Vibrio cholerae* O1. *Trends Microbiol* 23: 479 – 489
188. Askora A, Kawasaki T, Fujie M, Yamada T (2011) Resolvase-like serine recombinase mediates integration/excision in the bacteriophage phiRSM. *J Biosci Bioeng* 111: 109 – 116
189. Adams MJ, Lefkowitz EJ, King AM, Harrach B, Harrison RL, Knowles NJ, Kropinski AM, Krupovic M, Kuhn JH, Mushegian AR *et al* (2017) 50 years of the international committee on taxonomy of viruses: progress and prospects. *Arch Virol* 162: 1441 – 1446
190. Lefkowitz EJ, Dempsey DM, Hendrickson RC, Orton RJ, Siddell SG, Smith DB (2018) Virus taxonomy: the database of the international committee on taxonomy of viruses (ICTV). *Nucleic Acids Res* 46: D708 – D717
191. Adriaenssens EM, Krupovic M, Knezevic P, Ackermann HW, Barylski J, Brister JR, Clokie MR, Duffy S, Dutilh BE, Edwards RA *et al* (2017) Taxonomy of prokaryotic viruses: 2016 update from the ICTV bacterial and archaeal viruses subcommittee. *Arch Virol* 162: 1153 – 1157
192. Aiewsakun P, Adriaenssens EM, Lavigne R, Kropinski AM, Simmonds P (2018) Evaluation of the genomic diversity of viruses infecting bacteria, archaea and eukaryotes using a common bioinformatic platform: steps towards a unified taxonomy. *J Gen Virol* 99: 1331 – 1343
193. Simmonds P, Adams MJ, Benko M, Breitbart M, Brister JR, Carstens EB, Davison AJ, Delwart E, Gorbalenya AE, Harrach B *et al* (2017) Consensus statement: virus taxonomy in the age of metagenomics. *Nat Rev Microbiol* 15: 161 – 168

194. Simmonds P, Aiewsakun P (2018) Virus classification - where do you draw the line? *Arch Virol* 163: 2037 – 2046
195. Rakonjac J, Russel M, Khanum S, Brooke SJ, Rajic M (2017) Filamentous phage: structure and biology. *Adv Exp Med Biol* 1053: 1 – 20
196. Lorenz SH, Jakob RP, Weininger U, Balbach J, Dobbek H, Schmid FX (2011) The filamentous phages fd and IF1 use different mechanisms to infect *Escherichia coli*. *J Mol Biol* 405: 989 – 1003
197. Peeters BP, Peters RM, Schoenmakers JG, Konings RN (1985) Nucleotide sequence and genetic organization of the genome of the N-specific filamentous bacteriophage IKe. Comparison with the genome of the F-specific filamentous phages M13, fd and f1. *J Mol Biol* 181: 27 – 39
198. Khatoon H (1976) Genetic properties of RM 98, an R plasmid of Salmonella which determines sensitivity to the phage IKe. *Zentralbl Bakteriol Orig A* 234: 294 – 304
199. Bradley DE, Coetzee JN, Hedges RW (1983) IncI2 plasmids specify sensitivity to filamentous bacteriophage IKe. *J Bacteriol* 154: 505 – 507
200. Stassen AP, Schoenmakers EF, Yu M, Schoenmakers JG, Konings RN (1992) Nucleotide sequence of the genome of the filamentous bacteriophage I2-2: module evolution of the filamentous phage genome. *J Mol Evol* 34: 141 – 152
201. Chouikha I, Charrier L, Filali S, Derbise A, Carniel E (2010) Insights into the infective properties of YpfPhi, the *Yersinia pestis* filamentous phage. *Virology* 407: 43 – 52
202. Davis BM, Waldor MK (2000) CTXphi contains a hybrid genome derived from tandemly integrated elements. *Proc Natl Acad Sci USA* 97: 8572 – 8577
203. Waldor MK, Mekalanos JJ (1996) Lysogenic conversion by a filamentous phage encoding cholera toxin. *Science* 272: 1910 – 1914
204. Davis BM, Kimsey HH, Kane AV, Waldor MK (2002) A satellite phage-encoded antirepressor induces repressor aggregation and cholera toxin gene transfer. *EMBO J* 21: 4240 – 4249
205. Faruque SM, Asadulghani Kamruzzaman M, Nandi RK, Ghosh AN, Nair GB, Mekalanos JJ, Sack DA (2002) RS1 element of *Vibrio cholerae* can propagate horizontally as a filamentous phage exploiting the morphogenesis genes of CTXphi. *Infect Immun* 70: 163 – 170
206. Ikema M, Honma Y (1998) A novel filamentous phage, fs-2, of *Vibrio cholerae* O139. *Microbiology* 144(Pt 7): 1901 – 1906
207. Wang Q, Kan B, Wang R (2013) Isolation and characterization of the new mosaic filamentous phage VFJ Phi of *Vibrio cholerae*. *PLoS ONE* 8: e70934
208. Faruque SM, Bin Naser I, Fujihara K, Diraphat P, Chowdhury N, Kamruzzaman M, Qadri F, Yamasaki S, Ghosh AN, Mekalanos JJ (2005) Genomic sequence and receptor for the *Vibrio cholerae* phage KSF-1phi: evolutionary divergence among filamentous vibriophages mediating lateral gene transfer. *J Bacteriol* 187: 4095 – 4103
209. Nasu H, Iida T, Sugahara T, Yamaichi Y, Park KS, Yokoyama K, Makino K, Shinagawa H, Honda T (2000) A filamentous phage associated with recent pandemic *Vibrio parahaemolyticus* O3:K6 strains. *J Clin Microbiol* 38: 2156 – 2161
210. Chang B, Taniguchi H, Miyamoto H, Yoshida S (1998) Filamentous bacteriophages of *Vibrio parahaemolyticus* as a possible clue to genetic transmission. *J Bacteriol* 180: 5094 – 5101
211. Nakasone N, Honma Y, Toma C, Yamashiro T, Iwanaga M (1998) Filamentous phage fs1 of *Vibrio cholerae* O139. *Microbiol Immunol* 42: 237 – 239
212. Hill DF, Short NJ, Perham RN, Petersen GB (1991) DNA sequence of the filamentous bacteriophage Pf1. *J Mol Biol* 218: 349 – 364
213. Nazik H, Joubert LM, Secor PR, Sweere JM, Bollyky PL, Sass G, Cegelski L, Stevens DA (2017) Pseudomonas phage inhibition of *Candida albicans*. *Microbiology* 163: 1568 – 1577
214. Luiten RG, Putterman DG, Schoenmakers JG, Konings RN, Day LA (1985) Nucleotide sequence of the genome of Pf3, an IncP-1 plasmid-specific filamentous bacteriophage of *Pseudomonas aeruginosa*. *J Virol* 56: 268 – 276
215. Penner JC, Ferreira JA, Secor PR, Sweere JM, Birukova MK, Joubert LM, Haagensen JA, Garcia O, Malkovskiy AV, Kaber G *et al* (2016) Pf4 bacteriophage produced by *Pseudomonas aeruginosa* inhibits *Aspergillus fumigatus* metabolism via iron sequestration. *Microbiology* 162: 1583 – 1594
216. Mooij MJ, Drenkard E, Llamas MA, Vandenbroucke-Grauls CM, Savelkoul PH, Ausubel FM, Bitter W (2007) Characterization of the integrated filamentous phage Pf5 and its involvement in small-colony formation. *Microbiology* 153: 1790 – 1798
217. Klockgether J, Munder A, Neugebauer J, Davenport CF, Stanke F, Larbig KD, Heeb S, Schock U, Pohl TM, Wiehlmann L *et al* (2010) Genome diversity of *Pseudomonas aeruginosa* PAO1 laboratory strains. *J Bacteriol* 192: 1113 – 1121
218. Yang F, Pecina DA, Kelly SD, Kim SH, Kemner KM, Long DT, Marsh TL (2010) Biosequestration via cooperative binding of copper by *Ralstonia pickettii*. *Environ Technol* 31: 1045 – 1060
219. Tseng YH, Lo MC, Lin KC, Pan CC, Chang RY (1990) Characterization of filamentous bacteriophage phi Lf from *Xanthomonas campestris* pv. campestris. *J Gen Virol* Pt 71(8): 1881 – 1884
220. Dai H, Chiang KS, Kuo TT (1980) Characterization of a new filamentous phage Cf from *Xanthomonas-Citri*. *J Gen Virol* 46: 277 – 289
221. Kuo TT, Tan MS, Su MT, Yang MK (1991) Complete nucleotide sequence of filamentous phage Cf1c from *Xanthomonas campestris* pv. citri. *Nucleic Acids Res* 19: 2498
222. Dai H, Tsay SH, Kuo TT, Lin YH, Wu WC (1987) Neolysogenization of *Xanthomonas campestris* pv. citri infected with filamentous phage Cf16. *Virology* 156: 313 – 320
223. Ahmad AA, Askora A, Kawasaki T, Fujie M, Yamada T (2014) The filamentous phage XacF1 causes loss of virulence in *Xanthomonas axonopodis* pv. citri, the causative agent of citrus canker disease. *Front Microbiol* 5: 321
224. Ahmad AA, Kawabe M, Askora A, Kawasaki T, Fujie M, Yamada T (2017) Dynamic integration and excision of filamentous phage XacF1 in *Xanthomonas citri* pv. citri, the causative agent of citrus canker disease. *FEBS Open Bio* 7: 1715 – 1721
225. Meyer J, Brissac T, Frapy E, Omer H, Euphrasie D, Bonavita A, Nassif X, Bille E (2016) Characterization of MDAPhi, a temperate filamentous bacteriophage of *Neisseria meningitidis*. *Microbiology* 162: 268 – 282
226. Piekarowicz A, Klyz A, Majchrzak M, Szczesna E, Piechucki M, Kwiatek A, Maugel TK, Stein DC (2014) Neisseria gonorrhoeae filamentous phage NgoPhi6 is capable of infecting a variety of Gram-negative bacteria. *J Virol* 88: 1002 – 1010
227. Liu J, Liu Q, Shen P, Huang YP (2012) Isolation and characterization of a novel filamentous phage from *Stenotrophomonas maltophilia*. *Arch Virol* 157: 1643 – 1650
228. Liu J, Chen P, Zheng C, Huang YP (2013) Characterization of maltocin P28, a novel phage tail-like bacteriocin from *Stenotrophomonas maltophilia*. *Appl Environ Microbiol* 79: 5593 – 5600
229. Petrova M, Shcherbatova N, Kurakov A, Mindlin S (2014) Genomic characterization and integrative properties of phiSMA6 and phiSMA7, two novel filamentous bacteriophages of *Stenotrophomonas maltophilia*. *Arch Virol* 159: 1293 – 1303

230. Yu ZC, Chen XL, Shen QT, Zhao DL, Tang BL, Su HN, Wu ZY, Qin QL, Xie BB, Zhang XY *et al* (2015) Filamentous phages prevalent in *Pseudoalteromonas* spp. confer properties advantageous to host survival in Arctic sea ice. *ISME J* 9: 871 – 881
231. Jian H, Xiao X, Wang F (2013) Role of filamentous phage SW1 in regulating the lateral flagella of *Shewanella piezotolerans* strain WP3 at low temperatures. *Appl Environ Microbiol* 79: 7101 – 7109
232. Pederson DM, Welsh LC, Marvin DA, Sampson M, Perham RN, Yu M, Slater MR (2001) The protein capsid of filamentous bacteriophage PH75 from *Thermus thermophilus*. *J Mol Biol* 309: 401 – 421
233. Chopin MC, Rouault A, Ehrlich SD, Gautier M (2002) Filamentous phage active on the gram-positive bacterium *Propionibacterium freudenreichii*. *J Bacteriol* 184: 2030 – 2033
234. Kim AY, Blaschek HP (1991) Isolation and characterization of a filamentous viruslike particle from *Clostridium acetobutylicum* NCIB 6444. *J Bacteriol* 173: 530 – 535
235. Cantalupo PG, Calgua B, Zhao G, Hundesa A, Wier AD, Katz JP, Grabe M, Hendrix RW, Girones R, Wang D *et al* (2011) Raw sewage harbors diverse viral populations. *MBio* 2: e00180 – 11
236. Capella-Gutierrez S, Silla-Martinez JM, Gabaldon T (2009) trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 25: 1972 – 1973
237. Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 30: 772 – 780
238. Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30: 1312 – 1313

