Targeting mechanisms of tailed bacteriophages

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Abstract | Phages differ substantially in the bacterial hosts that they infect. Their host range is determined by the specific structures that they use to target bacterial cells. Tailed phages use a broad range of receptor-binding proteins, such as tail fibres, tail spikes and the central tail spike, to target their cognate bacterial cell surface receptors. Recent technical advances and new structure–function insights have begun to unravel the molecular mechanisms and temporal dynamics that govern these interactions. Here, we review the current understanding of the targeting machinery and mechanisms of tailed phages. These new insights and approaches pave the way for the application of phages in medicine and biotechnology and enable deeper understanding of their ecology and evolution.

Phages
Phages, or bacteriophages, are viruses that specifically infect bacteria for survival and replication.

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Like other viruses, phages depend on their host for the production and release of new viral particles. All phages encapsulate their genome in the proteinaceous capsid[1,2], and many phages have a tail attached to the capsid[3]. Phage tails are molecular machines that specifically recognize bacterial host cells, penetrate the cell envelope and deliver the phage genome into the cytoplasm. All tailed phages have a DNA genome and belong to the order of *Caudovirales* (from 'cauda', which is Latin for tail). Morphologically, they have traditionally been classified further into three families: the *Myoviridae*, *Siphoviridae* and *Podoviridae*. Myophages have long, contractile tails, siphophages have long, non-contractile tails, and podophages have short, non-contractile tails (FIG. 1). Members of the *Caudovirales* can infect a wide variety of bacterial hosts, and they are prevalent in many environments.

The tail structures are key determinants of the host specificity and infection process of the respective phages[4–6]. Many tailed phages use receptor-binding proteins (RBPs) at the distal end of their tail to interact with receptors on the bacterial cell surface. Tail fibres, tail spikes and tail tips function as RBPs, and they specifically recognize host receptors, such as lipopolysaccharide (LPS), teichoic acids and porins. Initial reversible attachment is followed by irreversible adsorption and ejection of the phage genome into the host cytoplasm.

The remarkable genetic diversity of both phage RBPs and bacterial receptors has made it difficult to study the mechanisms underlying their interactions. However, recent studies have provided a wealth of structural and mechanistic insights at single-molecule and atomic levels[6,7] and have inspired new or updated models of phage adsorption and recognition[4].

In this Review, we discuss the structural factors that govern the physical interaction of tailed phages with their cellular targets, which ultimately determine whether a phage will infect a bacterial host. Understanding the structural and molecular mechanisms of phage–host interactions is crucial for the application of phages in medicine and biotechnology (BOX 1). In addition, such knowledge will also provide deeper mechanistic insight into phage ecology and evolution, as well as their role in shaping the human microbiome and microbial ecology in general[8,9].

Phage structure and host recognition

The long-tailed myophages and siphophages and the short-tailed podophages (FIG. 1; TABLE 1) not only show great structural diversity but also use different mechanisms to interact with host cells[4–6].

Myoviridae. The T4 and Mu phages infect enterobacteria and have long, contractile tails[10] (FIG. 1a). The tail tube is attached to the capsid and, at the other end, to the baseplate complex. It is surrounded by a contractile sheath that facilitates puncturing of the bacterial cell envelope and delivery of the viral DNA[10]. The interaction between phage RBPs, that is, tail fibres, tail spikes and the central tail spike, and the bacterial surface triggers contraction[7].

Contractile tails are the most complex structures used for the delivery of phage genomes. Host recognition and adsorption of T4 are the best characterized examples in the *Myoviridae* family[4]. The T4 baseplate has both

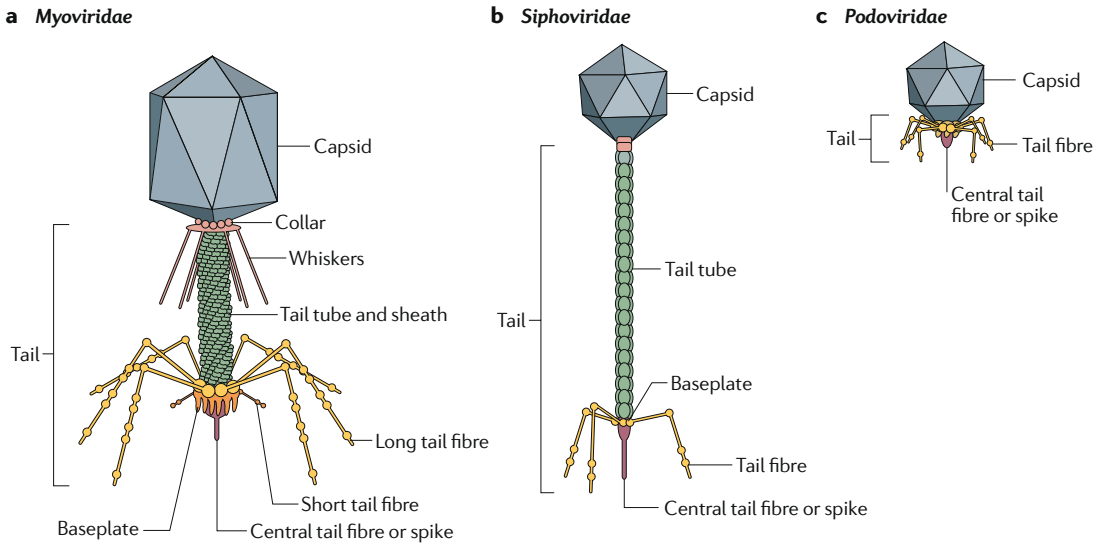


Fig. 1 | **Representative structures of tailed phages.** All tailed phages have a capsid that encloses and protects the genome and connects to the tail. **a** | Phages in the *Myoviridae* family are the only tailed phages with a contractile tail sheath. **b** | Both phages belonging to the *Myoviridae* and *Siphoviridae* families have a baseplate at the distal end of the tail to which receptor-binding proteins (RBPs), such as tail fibres and tail spikes, are attached. **c** | Because members of the *Podoviridae* have no baseplate, the RBPs directly attach to the tail. *Siphoviridae* and *Podoviridae* additionally have a central tail fibre or spike that protrudes from the distal end of the tail or baseplate.

short (gp12) and long (gp34–gp37) tail fibres[11] (FIG. 1a). The previous binding model assumed that the long tail fibres extend when T4 lands on the host surface and that the baseplate descends only after binding of all these fibres to their receptor[12]. Recent cryo-electron tomography evidence, however, has contradicted this model and revealed that the six long tail fibres alternate between folded and extended conformations[4,13] (FIG. 2a). An extended long tail fibre makes the initial contact with its specific host receptor, that is, LPS or outer membrane protein C (OmpC) (FIG. 2b). This is immediately followed by the contact of a second long tail fibre before the first has dissociated. The repetition of this process using different transiently extended long tail fibres enables the phage to move and find an optimal site for irreversible adsorption and genome ejection[4] (FIG. 2c). Reversible binding is the first step that determines the host range of T4 (REF.[14]).

When two or three of the six long tail fibres bind their receptors, a signal is transmitted to the baseplate, and it changes conformation[4], which enables some short tail fibres to irreversibly bind their receptor, that is, the outer core region of LPS. After further conformational change and expansion of the baseplate, the remaining short tail fibres bind and orient the virion for DNA ejection (FIG. 2d). Contraction of the sheath (FIG. 2e) is a rapid process that pushes the tail tube through the bacterial outer membrane[4] (FIG. 2f). Recently, atomic models showed that the tail assembly and capsid translate along and rotate about the tail tube axis[15]. The combination of translation and rotation enables the tip of the tail tube to effectively pierce the host membrane.

Notably, the T4 baseplate is more complex than the baseplates of most other contractile tailed phages. On the basis of genome data from contractile tailed phages and prophages, most have simple baseplates resembling the baseplate of myophage Mu, although they share the baseplate hub component, spike protein and wedge subunit with the T4 baseplate[16]. This finding led to the hypothesis that all contractile-tail-derived structures have evolved from these simple baseplates. Similarities also exist between the Mu-like simple baseplates and type VI secretion systems (T6SSs), phage-like translocation structures and R-type pyocins[16], which suggests that the contractile machinery is a versatile and widespread cell-penetrating device (BOX 2).

The mechanisms of host adsorption that myophages use to infect Gram-positive bacteria have also been described. Similar to T4, the staphylococcal phage phi812 contacts the cell surface with its tail fibres, which causes a conformational change of the baseplate[17]. However, the conformation of the phi812 baseplate is distinct from that of T4; it has thick 'legs', which comprise a trimer of RBPs and a trimer of elongated 'tripod' proteins. Together, these trimers form a cone-shaped receptor–tripod complex. Upon binding, the receptor–tripod complexes rotate 210°, which leads to the reorganization of the baseplate proteins into two layers parallel to the cell wall and exposes the RBPs[17]. The double-layered baseplate architecture of phi812 has been observed in other myophages that infect both Gram-positive[18] and Gram-negative bacteria[19,20]. Reorganization of the baseplate induces structural changes of the tail sheath and causes contraction of the sheath and DNA ejection[17].

Siphoviridae. Siphophages, such as the enterobacterial phages λ and T5, have long, flexible, non-contractile tails without a sheath (FIG. 1b). Some siphophages that infect Gram-positive bacteria have a baseplate-like structure similar to, but not as complex as, the baseplate of myophages. Siphophages that infect Gram-negative bacteria have no baseplate-like structure. Siphophages that target protein receptors on Gram-positive bacteria have

Porins
Proteins that assemble together to form a complex that acts as a channel (pore) across membranes through which molecules can diffuse.

Baseplate
A protein structure found in tailed phages from the *Myoviridae* and *Siphoviridae* families. Tails and tail appendages, including tail fibres or tail spikes, attach to the baseplate.

Prophages
Stable (relatively) forms of phage in which the genome is integrated into, and replicated with, the genome of the bacterial host, without lysis of the bacterial cell; prophages may also exist as extrachromosomal plasmids.

Box 1 | **Tools and applications of phages**

Phage therapy is one of the most promising alternatives to antibiotics, particularly for multidrug-resistant bacteria. The recent compassionate use of phages in humans in whom antibiotic therapy has failed has met with remarkable success[132,133]. However, the path to phage-based therapies in humans is still unclear, particularly in regard to regulation, safety and efficacy[134]. A detailed understanding of phage–host interactions will be relevant for the creation of tailored phages for therapy and will help take advantage of the recent advances in synthetic biology[135]. The same rationale applies to the use of phages in non-clinical settings, in which fewer regulatory hurdles may exist, including veterinary medicine[136], agriculture[137], aquaculture[138] and the food industry[139].

Phages have also been used to detect bacteria owing to their sensitive and specific interaction with the host. Some assays have used the detection of bacterial intracellular components after lysis by phages or detection of the resultant environment changes[140] as a marker of bacterial presence[141]. Others use phage propagation as a signal for the presence of the target bacteria[142,143]. The capacity of temperate phages to integrate in the bacterial genome has also been used in detection tools by engineering the phage with a reporter gene that generates a detectable signal once inside the bacterial cell[144]. Alternative approaches have immobilized phages on solid substrates to create a sensing layer of high bacterial specificity[145–147]. Others have bound phages to specific microparticles or nanoparticles and used these bioconjugates as tools to either detect bacteria directly[148] or separate bacteria from complex samples for subsequent identification[149,150].

Receptor-binding proteins (RBPs) have also been used as bacterial detection tools in manners similar to those described above[114,115,151]. More recently, RBPs have been suggested as O-serotyping probes to distinguish different bacterial glucosylation phenotypes[152]. Depolymerases encoded by phage RBPs have also been suggested for use in several applications that require species-specific targeting. For example, depolymerases may be used as adjuvants of the host immune system against bacterial infections by depriving bacteria of a major virulence factor, the capsule[91,153]. Alternatively, the extraordinary specificity of depolymerases for capsule types can be harnessed for diagnosis and typing[154,155]. A comprehensive understanding of all structural and molecular mechanisms governing the interactions of phages and their RBPs with the bacterial host will substantially improve phage-based tools for medical and biotechnological applications.

a straight tail fibre or spike directly attached to the tail or baseplate, whereas siphophages that target host polysaccharide receptors use side fibres with a high number of saccharide-binding domains[21]. Siphophages that infect Gram-negative bacteria generally have both a central, straight fibre and side fibres.

The RBP in T5 is pb5, and one pb5 molecule is located at the tip of the pb4 central fibre[22]. Interaction of pb5 with its specific receptor, ferrichrome outer membrane transporter/phage receptor (FhuA), triggers DNA ejection[23] (FIG. 3a). The tape measure protein pb2 creates a pore in the bacterial cytoplasmic membrane for DNA transfer[22].

Phage T5 has three additional L-shaped tail fibres that consist of pb1 and attach to the thin collar at the upper end of the conical basal structure[24]. The pb1 fibres increase adsorption to bacteria that carry O-antigens. In T5-like phages, such as coliphages DT57C and DT571/2, this binding is required for the central fibre to successfully reach vitamin B_{12} transporter BtuB, its host receptor on the outer membrane[25]. In contrast to T5, which has a single gene encoding L-shaped tail fibres, these phages have two genes, *ltfA* and *ltfB*. Three of the seven sequenced and annotated genomes of T5-like phages also use two genes to encode L-shaped fibres with distinct domains that recognize different O-antigen types and expand the phage's host range[25]. As only a few T5-like genomes are available so far, it is unclear whether a single-gene or dual-gene adhesin arrangement predominates among these phages. Nevertheless, the double gene arrangement of the tail fibres can be found in podophages, such as the enterobacterial phage K1-5 (REF.[26]), and myophages, such as the enterobacterial phage G7C[27].

It is less well understood how siphophages infect Gram-positive bacteria, but recent discoveries on phages that infect lactococci have provided interesting insights. Most lactococcal phages have a baseplate with multiple RBPs[28] (FIG. 3a). In their unbound state, the gp18 RBPs of siphophage p2 are retracted and recognize their host receptors laterally. This conformation results in a relatively low affinity owing to the limited number of exposed binding sites. Initial host recognition causes conformational changes in the baseplate and extension of the RBPs and exposure of all binding sites. When all RBP-binding sites are engaged, p2 binds irreversibly and ejects its DNA[29,30]. Siphophages that infect Gram-positive bacteria tend to have more complex baseplates with multiple RBPs that attach to different saccharide receptors on the host surface[31,32] than siphophages that infect Gram-negative bacteria. Nevertheless, siphophages of Gram-positive bacteria seem to have the same host range limitations as siphophages of Gram-negative bacteria. It appears that the structural differences between phages that infect Gram-positive and Gram-negative bacteria reflect the different host receptors that they target. This is most likely the reason why no phages have been found so far that infect both Gram-positive and Gram-negative bacteria, together with a lack of broad testing of host ranges.

Podoviridae. Podophages, such as coliphage T7 and *Salmonella* spp. phage P22, have short, non-contractile tails that consist of an upper tail adaptor protein that connects the tail to the capsid and a lower nozzle that is surrounded by six or twelve tail fibres or tail spikes[33] (FIG. 1c), but they have no baseplate. The first interaction of T7, the hallmark example of a podophage, involves host membrane proteins and facilitates further interaction of the gp17 tail fibres with LPS. This causes a conformational change in the tail fibres that orients them perpendicular to the cell surface and triggers protein and DNA ejection[6]. The ejected proteins presumably extend the short T7 tail and form a channel through which DNA enters the cell cytoplasm[6].

The adsorption machinery of P22 differs from that of T7. Instead of tail fibres, P22 has six gp9 tail spikes, which are connected to the tail tube and determine host range[34]. Their endoglycosidase activity is responsible for the degradation of the O-antigen receptor[35]. The end of the P22 tail is closed by a protein, which prevents DNA from exiting the capsid before binding. This protein is also thought to have alternative functions in host envelope penetration and triggering of DNA ejection[36,37]. Additionally, similar to T7, ejected proteins form a channel for DNA entry[38].

Podophages of Gram-positive bacteria, such as *Bacillus* spp. phage phi29, have similar mechanisms of adsorption and infection as podophages of Gram-negative bacteria. Similar to P22, phi29 has tail spikes attached to the tail tube, although there are twelve gp12

O-antigens
Repetitive glycan polymers that constitute the outermost domain of the lipopolysaccharide of Gram-negative bacteria.

Table 1 | **Host–phage interactions mediating adsorption of tailed phages**

Phage family	Adsorption module	RBP	Bacterial target	Host receptor	Example phage	Refs
Siphoviridae	Long non-contractile tail	Tail fibre	GN	O-antigen (smooth LPS)	T5	170
				Core oligosaccharide (rough LPS)	SSU5	96
				Proteins (for example, LamB, TolC and FepA)	λ, TLS and H8	102,171,172
				Flagella	Chi and iEPS5	112,173
				Pili	DMS3	174
			GP	Teichoic acids	LL-H	175
		Tail spike	GN	Capsule	Vi-II	52
				LPS	9NA	176
			GP	Protein (for example, YueB)	SPP1	104,177
		Central tail fibre	GN	Proteins (for example, BtuB and FhuA)	BF23, T5	178,179
		Tail tip	GN	Protein (for example, TonB)	H8	102
		Capsid filament	GN	Flagella	phiChi13 and phiCbK	111
Myoviridae	Long contractile tail	Tail fibre	GN	LPS	Mu	180
				Proteins (for example, OmpC and OmpF)	T4 and T2	58,181
				Flagella	SPN3US	182
			GP	Protein (for example, GamR)	γ	107
				Teichoic acids	A511	183
				Peptidoglycan	A511	183
		Tail spike	GN	Capsule	MAM1	184
				LPS	Det7	50
Podoviridae	Short contractile tail	Tail fibre	GN	LPS	T3 and T7	6,185
				Proteins (for example, Ail and OmpF)	Yep-phi	97
				Type IV pili	MPK7	120
		Tail spike	GN	Capsule	phiK1-5	26
				O-antigen (smooth LPS)	P22	38
				Protein (for example, OmpA)	Sf6	186
			GP	Teichoic acids	phi29	40

Ail, attachment invasion locus protein; BtuB, vitamin B_{12} transporter BtuB; FepA, ferrienterobactin receptor; FhuA, ferrichrome outer membrane transporter/phage receptor; GamR, cell wall protein GamR; GN, Gram-negative; GP, Gram-positive; LamB, maltoporin; LPS, lipopolysaccharide; Omp, outer membrane protein; RBP, receptor-binding protein; TolC, outer membrane protein TolC.

spikes instead of six tail spikes as in P22 (REF.[39]). The gp12 tail spikes mediate irreversible attachment and can degrade cell wall teichoic acids[40]. Following binding, a protein with enzymatic activity located at the distal end of the tail degrades the peptidoglycan layer, which paves the way for genome ejection[41].

In summary, all tailed phages use the tail and associated RBPs to interact with the host and to create a channel through which the DNA enters the cell. Despite this common strategy, the protein machinery responsible for host adsorption has specific features depending on phage taxa. Such features can influence the phage host range. Ecological studies have suggested that myophages have broader host ranges than siphophages and podophages[42], possibly because of their sophisticated baseplate[43].

Receptor-binding proteins

The RBPs of tailed phages (FIG. 3b; TABLE 1) have a high genetic plasticity, which enables the phages to adapt to and evolve to infect new hosts.

Tail spikes. gp9 of P22 is one of the best characterized tail spike structures (FIG. 3b). It forms a stable homotrimer, and each individual protein has three domains. The amino (N)-terminal particle-binding domain attaches to the phage tail and consists of two central β-sheets with five and three strands that are connected by extensive loops[34]. The carboxy (C)-terminal receptor-binding domain mediates attachment to the O-antigen and has endorhamnosidase enzymatic activity that cleaves the O-antigen and exposes the outer membrane[44]. Each subunit of the receptor-binding domain contains a right-handed, parallel β-helix that associates side by side with the other β-helices in the trimer such that the axes of the helices are almost parallel to the axis of the whole trimer. Finally, a short linker peptide connects the particle-binding and the receptor-binding domains. This linker peptide has high conformational flexibility[34,45], which facilitates the engagement of several receptors[46]. Notably, at least three tail spike molecules need to bind for successful infection[35]. In addition, conformational changes of the linker signal to

Genetic plasticity
The alterable nature of genomes that enables the exchange of nucleic acids from one organism to another, usually to adapt to new environmental conditions.

β-helix
A protein structure formed by parallel polypeptide chains associated in a helical pattern with either two or three faces; may be left-handed or right-handed.

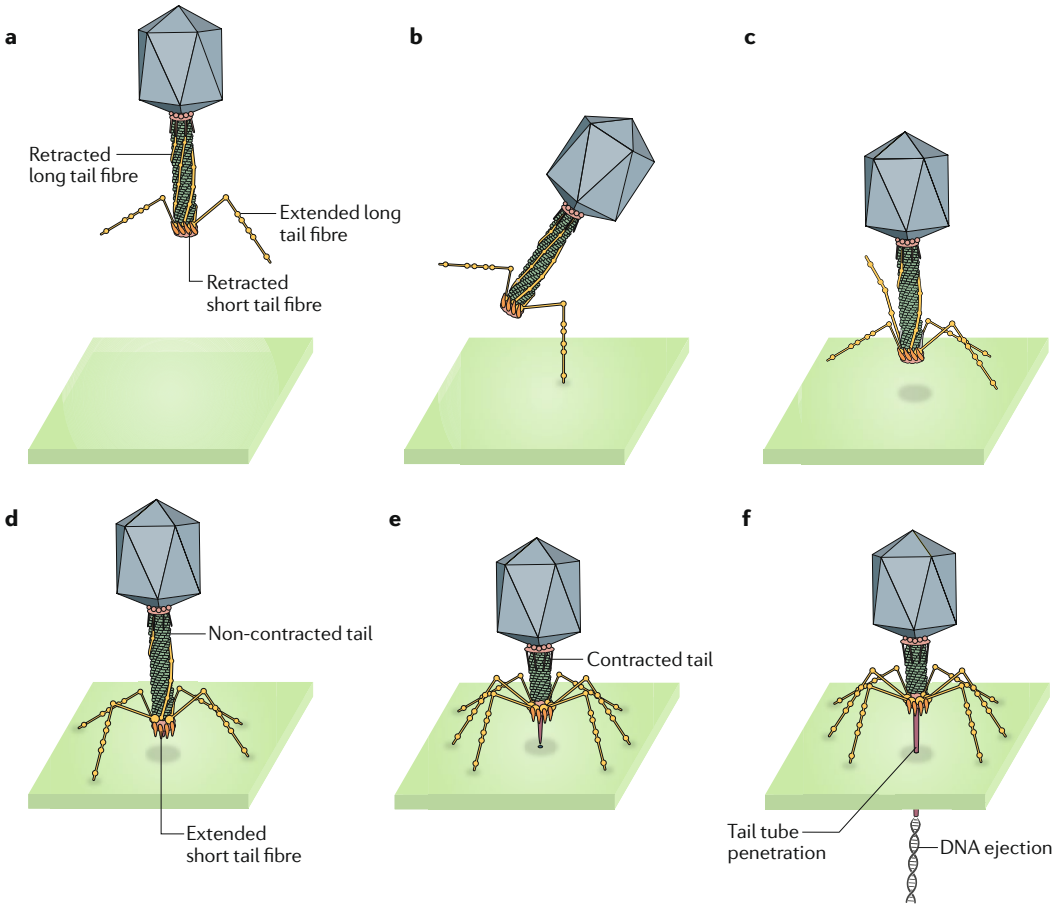


Fig. 2 | **Model of phage T4 adsorption to the host surface. a** | In its unattached state, the six long tail fibres of phage T4 extend and retract. **b** | An extended long tail fibre initiates contact with the bacterial cell surface. **c** | Binding of transiently extended long tail fibres allows phage T4 to move on the surface of the cell to find an optimal site for irreversible adsorption. **d** | Binding of two or three of the long tail fibres to their cellular receptor changes the baseplate conformation and releases the short tail fibres, which irreversibly bind to the host receptor. **e** | Irreversible binding causes the baseplate to assume a star configuration, triggering contraction of the tail and the release of the remaining long tail fibres, which also bind to the host. **f** | Tail contraction leads to the ejection of the phage DNA into the cell. Adapted with permission from REF.[4], Proceedings of the National Academy of Sciences of the United States of America.

Vi capsule
A bacterial capsule that exposes the Vi antigen, a heat-labile somatic antigen thought to be associated with virulence in some bacteria.

Deacetylation
The removal of an acetyl group from a chemical compound.

Recombination
The process by which genetic material is exchanged between DNA molecules.

Homotrimer
A protein composed of three identical units of polypeptide.

Monomer
An individual molecule that can associate with similar molecules to form a larger molecule (dimer, trimer and polymer).

the particle-binding domain[47] to trigger DNA ejection[34]. This linker is conserved among podophages.

Generally, P22-like phages have a conserved particle-binding domain but variable receptor-binding domains[48]. However, some P22-like phages, such as SP6, maintain the P22 receptor-binding domain with particle-binding domains of other phages[49]. Furthermore, the podophage SP6 and the myophage Det7 both infect *Salmonella* spp. and have similar tail spikes, gp49 and gp207, respectively, which suggests the transfer of receptor-binding domains between distinct phage taxa[50]. In *Pseudomonas* spp. phage LKA1, the tail spike protein contains an O5-serotype specific polysaccharide lyase[51].

Vi-like myophages also infect *Salmonella* spp. and have similar tail spikes as P22-like podophages[19,50] but target the Vi capsule[52]. Vi-like phages are morphologically diverse and occur in all three families of tailed phages. All their tail spikes contain a domain that mediates recognition and deacetylation of the Vi exopolysaccharide[53]. Vi-like phages also infect bacteria without a Vi capsule because they also have branched tail spike proteins, similar to the coliform K1 podophages, and RBPs with different host specificities. This larger host range increases the probability of recombination, including of the Vi-targeting RBPs, with non-Vi infecting phages during co-infection of a non-Vi host[52].

In summary, tail spikes have largely conserved structures but vary in their receptor-binding domains and the host specificities that they confer. Furthermore, tail spikes often carry enzymes that degrade the cell wall and facilitate DNA ejection (see below).

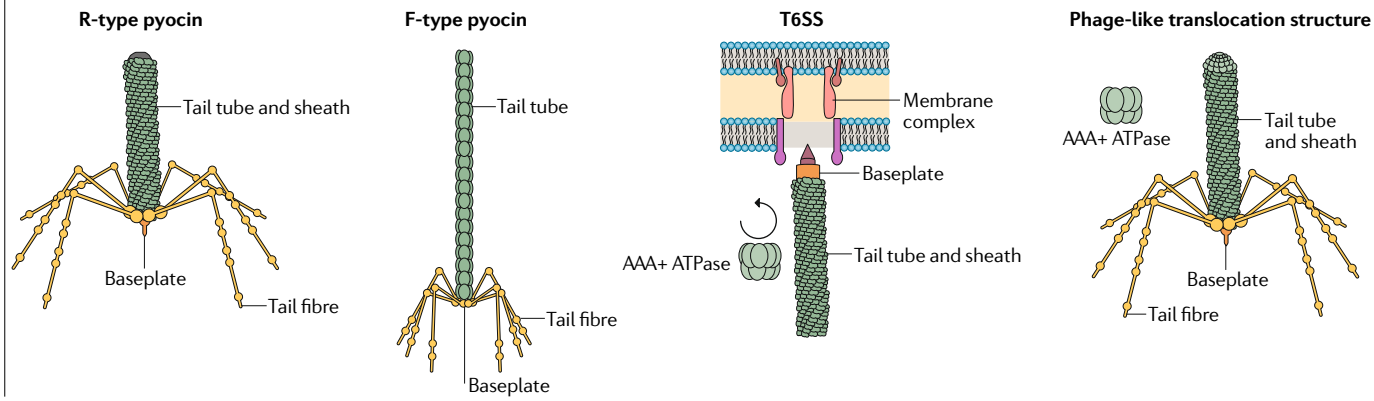
Tail fibres. In contrast to tail spikes, tail fibres usually have no enzymatic activity. The T4 long tail fibres, which are the best characterized tail fibres so far, are an assembly of four distinct proteins[54] that assume an articulated leg-like shape[55]. The proximal segment (the 'thigh') is composed of a homotrimer of gp34, and a hinge (the 'knee') formed by a monomer of gp35 (REF.[55]) connects it to the distal segment (the 'lower leg') consisting of a trimer of protein gp36 and a trimer of protein

Box 2 | **Bacterial phage tail-like complexes**

Phage tail-like complexes that are homologous to phage components but perform a different biological function have been identified in a broad range of bacteria. These structures are evolutionarily related to phages and share a conserved protein core (tail tube, tail spike, baseplate and often receptor-binding proteins (RBPs)) but lack the DNA-bearing capsid that is required for replication (see the figure). In contrast to phages, phage tail-like complexes have been shaped by evolution to enhance bacterial fitness. Three classes of phage tail-like complexes can be recognized. Phage tail-like bacteriocins (PTLBs) comprise R-type contractile complexes that derive from *Myoviridae*-like tails and non-contractile F-type complexes that are homologous to *Siphoviridae*-like tails[156].

PTLBs target both Gram-positive and Gram-negative bacteria[157] and kill by draining the proton motive force[158]. The phage tail-like translocation structures (PTLSs) are a second class of phage tail-like complexes. These systems are a widespread and functionally diverse group of *Myoviridae* phage-derived contractile complexes that target insect cells[79,159–161]. A third class is the type VI secretion systems (T6SSs), which are derived from *Myoviridae*[162] and have been identified in different bacterial species[163]. Unlike the other phage tail-like complexes, T6SSs are not deployed by cell lysis but rather are embedded in the cell envelope[164]. They kill competing bacteria or affect eukaryotic cells upon contact by injecting a spiked tube charged with cell-effector proteins[78,165].

The mechanism and dynamics of host recognition by phage tail-like complexes are still poorly understood and have so far been examined only for R-type PLTBs (pyocins), which bind to bacterial lipopolysaccharide (LPS) receptors[166]. On the basis of the structural similarity with contractile phages, it is likely that contraction of R-type pyocins is also triggered by binding of several fibres to the receptor, which then results in tube injection and draining of the ion-motive force[158]. Five pyocin subgroups (R1 to R5) have been distinguished in *Pseudomonas aeruginosa*, all of which possess tail fibres with a conserved amino terminus and a variable carboxyl terminus[167]. The variable region of the fibres governs the host range of pyocins by facilitating binding to different moieties (that is, L-Rha, α-Glc and β-Glc) of LPS[166]. The host range of R-type pyocins and other PLTBs has successfully been changed by replacing the carboxy-terminal part of the tail fibres with analogous parts from the tail fibres of phages[167,168]. This indicates that the transmission and interpretation of fibre-mediated cues for contraction occur similarly in R-type PLTBs and phages. An additional piece of information on host recognition by phage tail-like complexes comes from an electron cryotomography study of the T6SS of *Myxococcus xanthus*, which identified a tail fibre-like extracellular antenna that might trigger contraction in response to a still-unknown signal or receptor[164].



gp37 (FIG. 3b). gp37 also forms the needle-shaped 'foot', which contains the receptor-binding region at its C terminus. The proper trimeric assembly of the distal part of the long tail fibres requires the action of two phage-encoded chaperones[56].

The receptor-binding region of gp37 is an elaborate, interwoven trimer with a globular collar domain, an elongated needle domain and a head domain. The collar domain itself is a trimer in which each monomer comprises two antiparallel β-sheets and an α-helix. Conversely, the needle domain is a long, six-stranded, antiparallel β-barrel formed by residues from each of the three polypeptides, which complete one and a half turns around the fibre axis. Central to the interwoven strands are iron ions that hold the strands together[57]. The head domain contains the residues involved in host binding[57]. It is likely that aromatic and positively charged residues in this region bind LPS. Additionally, the head domain was recently suggested to bind to the centre of the OmpC trimer vertically, with its lateral surface interacting at multiples sites with the extracellular loops of the porin[58].

Sequence similarity of the receptor-binding region of T4 gp37, Tula and Tulb gp37 and λ Ur side tail fibres[59], with the exception of the head domains, indicates overall structural conservation superimposed by modular evolution of the head and thus host specificity[57].

The short T4 tail fibres have a simple triple-stranded β-helix structure[60]. They are composed of a single protein, gp12, which forms a parallel homotrimer[61] (FIG. 3b). The thin N-terminal part of the homotrimer attaches to the baseplate, whereas the arrow-shaped, globular C-terminal part binds the host receptors[60]. The C terminus has a 'knitted' fold formed by three intertwined strands[62]. This domain has a metal-binding site containing a zinc ion that is likely necessary for the assembly and stability of the trimer[60]. A chaperone is also required for correct folding of the trimer[63]. The short tail fibre is hinged with the receptor-binding region hidden in the baseplate and enables binding, and it rotates and extends in a process requiring the structural protein gp10 (REF.[64]).

In contrast to tail fibres of T4, tail fibre structures of the *Siphoviridae* and *Podoviridae* families have not been extensively characterized so far. In the siphophage T5, the L-shaped pb1 fibres are homotrimers composed of a β-helical distal domain with a bullet shape and a thin proximal domain that may have a collagen fold[65]. An intramolecular chaperone is required for proper folding of the C-terminal triple helix;

Chaperones
Proteins that interact with and assist in the folding and unfolding or assembly and disassembly of other proteins without being part of the final structure.

α-helix
A right-handed coiled conformation of proteins in which the resulting structure resembles a helix.

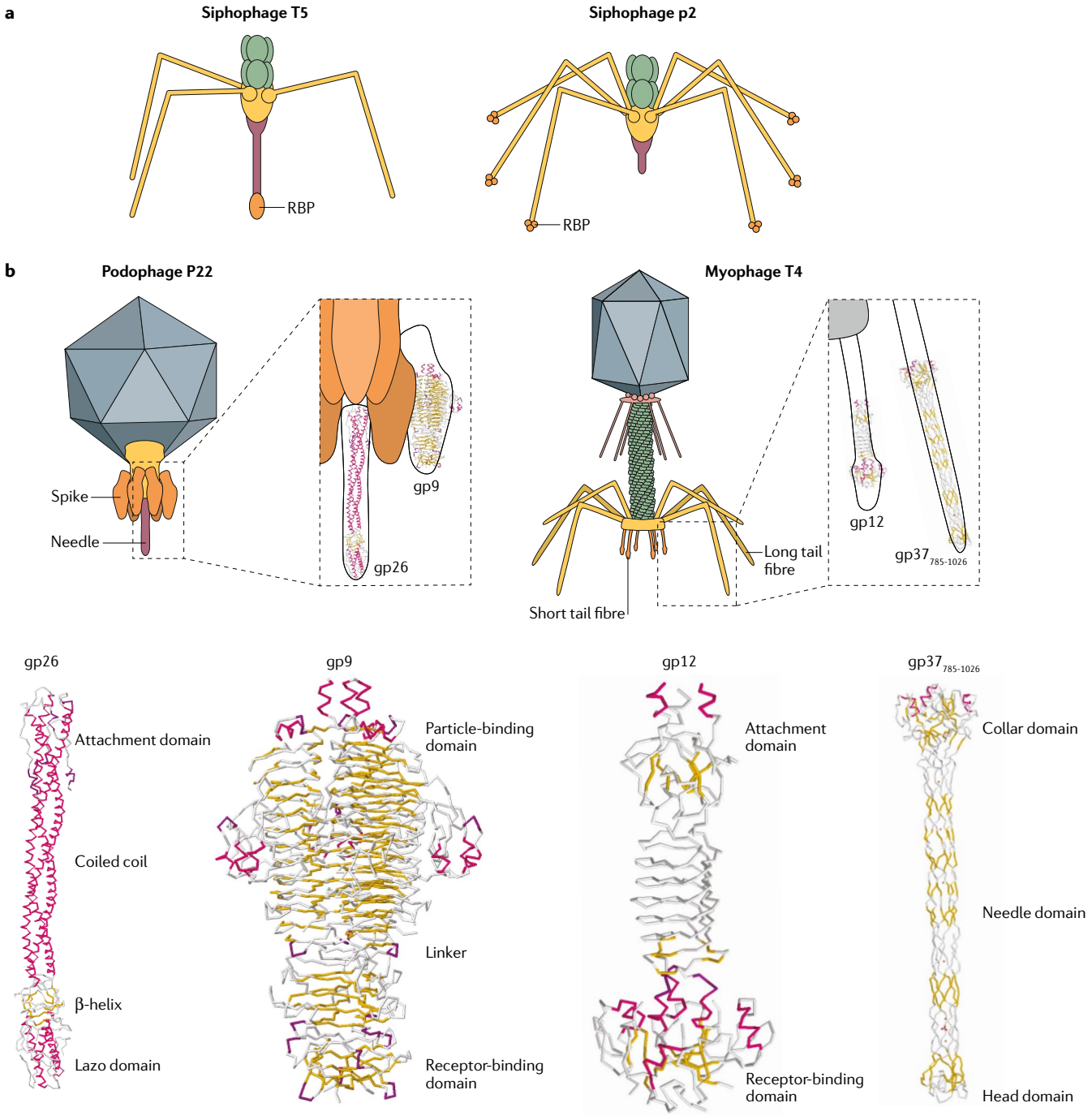


Fig. 3 | **Receptor-binding proteins of tailed phages. a** | Phages in the *Siphoviridae* family that infect Gram-negative bacteria (T5, left panel) and Gram-positive bacteria (p2, right panel) differ in their adsorption apparatus. Phage T5 has one receptor-binding protein (RBP) located at the tip of the straight central tail fibre, and three additional L-shaped tail fibres protrude from the baseplate. Phage p2 has 18 RBPs distributed on 6 tail fibres and a central short tail spike. **b** | The structures of some phage RBPs have been determined. Podophage P22 binds to its receptors through the g9 tail spike (Protein Data Bank (PDB): 1TSP)[169] and penetrates the host cell with its tail tip consisting of the gp26 needle protein (PDB: 2POH)[36]. The RBPs of myophage T4 are the long tail fibre tip gp37 (PDB: 2XGF)[57] and the short tail fibre gp12 (PDB: 1H6W)[60].

once folding is complete, proteolysis leads to the release of the chaperone domain[66]. The specific receptor-binding site remains unknown but is likely in the groove that is exposed after chaperone cleavage[67]. The crystal structure of the gp18 RBP from lactococcal phage p2 RBP resembles the gp12 homotrimer of myophage T4 (REF.[68]).

In podophage T7, the gp17 tail fibres are elongated homotrimers that do not use phage chaperones for folding. As observed for the tail fibres of some of the phages

Proteolysis
The breakdown (hydrolysis) of proteins or peptides into their key components, peptides and amino acids, by the action of enzymes.

Phage tail-like complexes
Protein complexes that are homologous to phage tails and facilitate the killing of cells or influence bacterial and eukaryotic cells through physical interactions.

described above, the particle-binding domain seems to be conserved among *Autographivirinae*[69], a *Podoviridae* subfamily, whereas the receptor-binding domains diverged to adapt to different hosts[70].

Taken together, tail fibres share the modular structure of tail spikes, with particle-binding and receptor-binding domains. However, they have generally longer and more complex structures than tail spikes, which often require chaperones for proper folding and functionality. Notably, although chaperones are not needed for folding of tail spikes, they are necessary for phage assembly. As with tail spikes, tail fibres have highly variable receptor-binding domains and are involved in reversible and/or irreversible adsorption. They have no enzymatic activity, although regions encoding depolymerases have been found in tail fibre genes[71]. Tail fibres are involved in triggering conformational changes that lead to DNA ejection from the phage capsid.

Membrane-penetrating proteins. The tip region of the tail is most likely involved in overcoming the cell wall for DNA ejection in all phages. For phages of Gram-positive bacteria, this involves penetration of a thick cell wall and an inner membrane, whereas phages of Gram-negative bacteria face the obstacles of a thinner wall but both an outer and inner cell membranes. Atomic resolution studies have revealed the structure and function of a few proteins involved in overcoming the outer membrane of Gram-negative bacteria, but little is known about how phages overcome the inner membrane.

The central spike protein gp5 of myophage T4 is a lysozyme that hydrolyses glycosidic bonds in peptidoglycan. It forms a stable complex with protein gp27 and resembles a long torch. The gp27 trimer forms the cylindrical part of the structure and encompasses three N-terminal domains of the trimeric gp5. Attached to these is the gp5 C-terminal domain, folded into a trimeric β-helix[11,72]. Adjacent to the helix are the three gp5 lysozyme domains, which are connected to the N-terminal and C-terminal domains by two long peptide linkers that run along the side of the helix[11]. These linkers prevent binding of the peptide portion of the substrate to the lysozyme domain, and the β-helix blocks the polysaccharide binding site. Thus, dissociation of the β-helix restores full lysozyme activity[11] and enables localized peptidoglycan digestion and thus facilitates penetration of the tail tube[73]. The mechanism for puncturing the inner membrane is still unclear, but it is hypothesized to involve gp27 or the ejected tape measure protein gp29 (REF.[4]).

Tape measure proteins are named after the role they play in defining tail length. pb2 and HK97 are the tape measure proteins of the enterobacterial siphophages T5 and HK97, respectively, and they are hypothesized to form a channel that bridges the outer and inner membranes of the host. pb2 has three domains: a coiled coil domain that spans the tail and serves as a sensor for triggering the opening of the head–tail connector, a transmembrane domain consisting of two α-helices, and a metallopeptidase motif[74]. The transmembrane and metallopeptidase domains are presumably located inside the straight fibre formed by pb5. Binding of pb5 to its host receptor FhuA is thought to trigger major conformational changes in pb2, causing the release of the DNA from the phage capsid. Simultaneously, the pb2 metallopeptidase degrades peptidoglycan, the outer and inner membranes fuse, and a pore for DNA transfer is formed[74]. It is unclear how pb2 fuses the membranes; by contrast, other siphophages, such as coliphage HK97, need host proteins for membrane penetration. Genetic analysis suggests that HK97 enters the periplasm, where it assembles into a conduit assisted by the host periplasmic chaperone FkpA. HK97 then interacts with the inner membrane glucose transporter protein PtsG and becomes anchored to both the outer and inner membranes, which enables passage of the phage DNA into the bacterial cytoplasm[75]. The needle protein gp26 of podophage P22 (FIG. 3b) is a homotrimeric left-handed twisted fibre similar to a podophage tail fibre. The N-terminal domain of the needle is formed by a hydrophobic moiety and a fibrous α-helical core and functions as a plug of the portal protein channel[76]. The C terminus contains a triple β-helix and a novel α-helical domain (termed the lazo domain), and it likely mediates cell envelope penetration[36]. An aromatic tyrosine residue is located at the extreme tip of the gp26 needle and likely initiates host contact by dipping into the outer membrane to initiate membrane fusion and trigger conformational changes. The P22 needle differs considerably from the T4 tail tip: it is largely α-helical, injected into the host and has no enzymatic activity[36]. It must be noted that the role of gp26 in outer membrane penetration is still highly speculative, and further studies are required to understand how the P22 tail overcomes both the outer and inner membrane.

As in P22, *Bacillus* spp. podophage Ø29 has a tail tip that can penetrate cells. However, this tail tip forms a 'knob' out of gp9 and gp13 (REF.[77]). Gp9 is a cylindrical, tube-like homohexamer with a β-barrel N-terminal domain, a central tube composed of an α/β-domain and a middle β-domain, and another β-domain at the tip. A hydrophobic long loop (L loop) protrudes from the inner wall near the tip β-domain and fills most of the interior of the tube. On exiting the tube, the L loop forms a cone-shaped tip that penetrates the membrane and forms a channel for DNA release into the cell[41]. In addition, the complex contains the gp13 enzyme for peptidoglycan degradation[77]. The membrane-penetrating proteins of phage tail-like complexes are homologous to those of phages, pierce the cell envelope and deliver a broad range of effector proteins. Examples include proteins that modify the membrane, degrade peptidoglycan or DNA, form membrane pores, deplete cellular $NAD(P)^+$ or rearrange the actin cytoskeleton[78,79].

Membrane-penetrating proteins are structurally and functionally distinct from tail fibres and tail spikes. Their main function is breaching of the cell boundary, although virtually nothing is known about penetration of the inner membrane. Moreover, membrane-penetrating proteins, for example, gpJ from λ phage[80], may also be involved in adsorption and determine the host range.

RBP phage-encoded enzymes. Tailed phages of both Gram-negative and Gram-positive bacteria commonly harbour peptidoglycan-degrading enzymes, including endopeptidases[81], *N*-acetylmuramyl-L-alanine amidases[82], *N*-acetylglycosaminidase[83,84], lysozymes[85] and lytic transglycosylases[86]. These enzymes locally degrade or rearrange peptidoglycan[87], and although they are not always required for infection, they provide advantages under particular conditions. For example, the peptidoglycan hydrolase in the tails of mycobacterial siphophage TM4, enterobacterial myophage T4 and lactococcal siphophage Tuc2009 was necessary for infection of cells in the stationary phase but not for cells growing exponentially[81,88,89].

Phages can also have enzymes that target other bacterial structures. The podophage F116 uses lyases to dissolve alginate exopolysaccharide that is secreted by *Pseudomonas* spp.[90]. Pectate lyases in the tail spike proteins of some phages might hydrolyse glycosidic bonds and thereby reduce bacterial virulence[91]. Vi phages of distinct taxa share an acetyl esterase that targets the acetyl modification on sugars[52]. Phages that infect bacteria with a polysaccharide capsule often encode endoglicosidases[48], endosialidases[92] and enzymes with antibiofilm activity[93].

Host receptors

The specificity of phage–host interactions is defined not only by the phage RBPs but also by the type and structure of the host receptors. Receptor localization, amount and density on the cell surface also influence specificity. Gram-positive and Gram-negative bacteria differ substantially in the thickness, uniformity and lipid and lipoprotein content of the cell wall and in the receptors available for phage adsorption (FIG. 4; TABLE 1).

Gram-negative bacterial receptors. The main receptor in Gram-negative bacteria is LPS, which occurs in both smooth and rough varieties. Although both forms possess core polysaccharides and lipid A, they differ in the presence (smooth) or absence (rough) of O-antigens. Furthermore, O-antigens differ between different host bacteria. The conserved nature of the LPS core suggests that phages targeting the core have greater potential for a broad host range[94]. Still, as observed for R-pyocins[95], it is possible that the O-antigen blocks binding to the LPS core, which implies that the core might not be an ideal target despite its conservation.

Phages recognize the O-antigen with their tail fibres[25] or tail spike proteins[51], and they can hydrolyse the O-antigen to enable penetration of the tail. Podophage T7 and siphophage SSU5 use the gp17 and gp22 tail fibres, respectively, to bind rough LPS *Shigella* spp., *Escherichia* spp. and *Salmonella* spp.[6,96]. Notably, the same phage can bind to different structures of the LPS core when interacting with different bacterial species and genera[94].

Some phages bind outer membrane porins, such as OmpC and OmpF[58,97]. For example, myophage T4 can use either OmpC or LPS for adsorption[58]. The long tail fibres, gp34 and gp37, bind both receptors; however, the top surface of the distal tip presumably interacts with LPS, whereas the lateral surface binds to OmpC[58]. Other transport proteins have also been used as receptors, including vitamin B_{12} uptake protein BtuB[98], maltoporin LamB[99], efflux pump TolC[100], ferrichrome transport protein FhuA[101] and TonB[102].

Gram-positive bacterial receptors. In Gram-positive bacteria, the main component of the cell wall is peptidoglycan, which is a heteropolymer of *N*-acetylglucosamine and *N*-acetylmuramic acid. A tetrapeptide is attached to the *N*-acetylmuramic acid through a hydroxyl group. The Gram-positive cell wall also contains teichoic acids, which are polyol phosphate polymers with either ribitol or glycerol linked by phosphodiester bonds. Bacterial species differ in the substituent groups on the polyol chains. For example, *Staphylococcus aureus* and *Bacillus subtilis* have similar peptidoglycan and teichoic acids structures, and phages specific for *S. aureus* may also attach to *B. subtilis*[103]. By contrast, the teichoic acid substituents differ, with D-glucose on *B. subtilis* and *N*-acetylglucosamine on *S. aureus*, and they determine phage specificity for each species[104–106]. Phages can also bind the backbone of the teichoic acid. Interestingly, in *S. aureus*, siphophages preferentially bind the substituent groups, whereas myophages prefer the backbone[105]. Furthermore, teichoic acid adsorption can be reversible, for example, in the *S. aureus* phages 3C, 52A, 71, 77 and 79, and irreversible adsorption also requires binding to the tetrapeptide chain in peptidoglycan[94], to the *O*-acetyl groups of the muramic acid in peptidoglycan[106] or to proteins. Indeed, phages can also bind proteins of Gram-positive bacteria, for example, the cell wall protein GamR in *Bacillus anthracis*[107], the extra-membrane domain of YueB in *Bacillus subtilis*[108] and the phage infection protein (Pip) in the membrane of *Lactococcus* spp.[109]. Phages can also bind a distinct cell wall polysaccharide of *Lactococcus lactis*, the pellicle. A semi-conserved core motif of the pellicle promotes initial phage binding, and differences in the rest of the pellicle polymer provide strain specificity[28]. Interestingly, bacteria can exchange cell surface components, and this transiently changes their sensitivity to phages[110]. This phenomenon was found in *B. subtilis* strains, in which the transfer of YueB receptors through membrane vesicles enabled phage SPP1 to infect a previously insensitive strain, but exchange is proposed to occur even at the interspecies level[110]. Fewer phage receptors have been found in Gram-positive bacteria than in Gram-negative bacteria. The difficulty in identifying phage receptors in the complex and dense cell walls of the former is the main cause of this discrepancy. It is expected that recent technological advances, such as single particle cryo-electron microscopy, will improve the identification of phage receptors in Gram-positive bacteria.

Flagella and pili. Appendages such as flagella and pili can also function as phage receptors. Binding to flagella and pili might increase the chance of phage attachment and infection in environments in which phages are unlikely to encounter their hosts[111]. Phages that use flagella as a receptor, such as iEPS5 (infecting *Salmonella* spp.)[112], phiCbK (infecting *Caulobacter* spp.)[111],

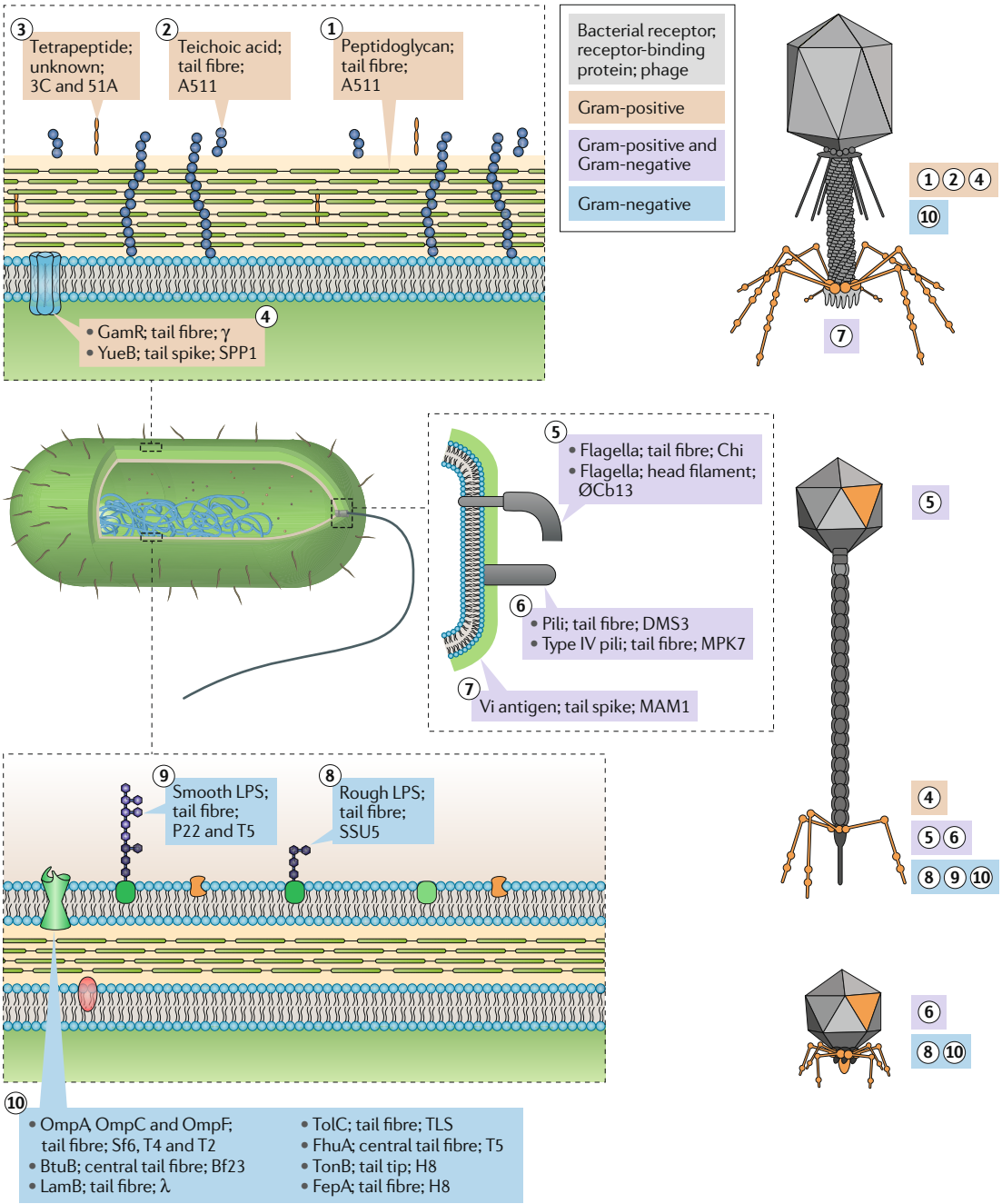


Fig. 4 | **Gram-negative and Gram-positive bacterial receptors for the attachment of tailed phages.** The legend for both bacterial receptors is given in the middle panel. The Gram-positive and Gram-negative cell walls and thus the structures available for phage binding differ substantially. Some of the main phage receptors in Gram-positive bacteria are peptidoglycan and teichoic acids. In Gram-negative bacteria, tailed phages bind lipopolysaccharide (LPS), both the rough form without an O-antigen and the smooth form with an O-antigen. Both Gram-negative and Gram-positive bacteria can have flagella, pili and Vi antigen, for example, those found in the capsule of *Salmonella* spp., represent potential phage receptors. Notably, phages often bind these latter bacterial structures with their head or the side of the tail fibre or spike to leave room for the other receptor-binding proteins to engage the ultimate bacterial receptors on the bacterial cell surface. Numbering on the left indicates the bacterial structure bound by the phage receptor-binding protein identified on the right with the same numbering. BtuB, vitamin B_{12} transporter BtuB; FepA, ferrienterobactin receptor; FhuA, ferrichrome outer membrane transporter/phage receptor; GamR, cell wall protein GamR; LamB, maltoporin; TolC, outer membrane protein TolC.

PBS1 (REF.[113]) and PBP1 (both infecting *Bacillus* spp.)[114], typically bind flagellin proteins, such as FliC, FliB and FliK, at the distal tip of the flagellum using their tail fibres. The phages are then transported by flagellum motility along the filament to their ultimate receptors[115,116]. Some phages, such as *Asticcacaulis biprosthecium* siphophages AcM4 and AcS2, specifically use the capsid and tail to bind the flagellum, which

Tropism
The orientation, by growth or movement, of all or part of an organism to an external stimulus.

leaves the distal part of the tail free for adsorption to the cell surface[117]. Interestingly, the tropism of phages seems to be restricted to lateral flagella. In fact, polar flagella might inhibit phage infection through their rotation in some bacteria[118].

Tailed phages can also attach to pili, a common mechanism of *Pseudomonas* spp. phages, such as phiKMV[119] and MPK7 (REF.[120]), which specifically bind to type IV pili. Although the phage–pili interaction has not yet been characterized at the atomic level, a recent cryo-electron microscopy reconstruction of two F family pili has provided some relevant clues[121]. Pili are assemblies of protein–phospholipid units, and the lipids facilitate pilus growth and retraction. This function is essential to bring attached phages closer to the membrane where the irreversible receptor is located. Moreover, a protein previously reported as a potential phage attachment site, the TraA pilin[122], was found to be located at the outside of the pilus filament, providing further evidence of its relevance for phage adsorption[121]. Furthermore, *Pseudomonas aeruginosa* has been shown to block phage adsorption by glycosylation of its type IV pili[123].

Capsule and slime layers. Some bacteria produce a polysaccharide capsule and/or a slime layer. These layers can block access to cell wall receptors but might contain receptors for phage adsorption. An example is the Vi antigen typically present on the capsules of *Salmonella* spp., *Citrobacter* spp. and *E. coli*. For example, the *Salmonella* spp. myophage ViO1 (REF.[52]) and the *Klebsiella* myophage o507-KN2-1 (REF.[124]) can bind the Vi antigen. The capsule might function as a primary receptor, whereas cell wall components mediate irreversible binding[94,125]. Phages infecting the *Acinetobacter baumannii–calcoaceticus* complex also target hosts according to capsule type. Interestingly, podophages targeting these bacteria seem to be highly similar and diverge mainly in the receptor-binding site of the tail fibres, which has depolymerase activity for specific capsule compositions[126]. ØNIT1 uses a different approach to target encapsulated *B. subtilis*. The capsule of *B. subtilis* subsp. *natto* is not anchored to peptidoglycan, and thus a few cells remain naked. These cells are the first target of the phage and are used to produce capsule-hydrolysing enzymes during the infection cycle. These enzymes are released together with the phage progeny after cell lysis to enable infection of the encapsulated cell population[127].

Conclusions

The structural determinants of phage–host interactions have been extensively characterized for a few tailed phages, but considering the vast diversity of tailed phages and their structures, many fascinating discoveries await. Although such diversity makes it difficult to predict associations between RBP structures and targeted host receptors, some trends emerge. RBPs have a modular structure, and combining different modules contributes to the evolution of RBP diversity. This mostly results from exchange of or mutations in the C-terminal domain of the RBP and often leads to a change in host range. The incredible variety of bacterial structures that phages use as receptors shows the evolutionary opportunism of phages and has led to distinct phage host specificities and range. Furthermore, phages from the same taxa or infecting the same bacterial species often target different receptors. Thus, finding and predicting associations between host receptors and phage specificity remain a major challenge. Recent advances in techniques such as cryo-electron microscopy[4,17,128,129] and single particle cryo-electron microscopy[7,130] will help to elucidate the molecular mechanisms of phage–host interactions. However, these techniques cannot fully take into account the dynamics involved in host attachment. Nonetheless, this can be addressed by resolving the structure of phages at different stages of attachment, such that the dynamics of individual phage features, such as the baseplate, can be inferred[7]. Understanding the mechanisms that govern the interactions of tailed phages with their host will provide the necessary basis for the use of phages as antimicrobials. Furthermore, this mechanistic knowledge combined with metagenomics helps elucidate the complex interplay of phages and bacteria in natural ecosystems and how they shape nutrient cycles and gene flow. Notably, recent evidence suggests that non-tailed phages are much more prevalent in the environment than previously assumed[131], and it will be essential to also elucidate the interactions of non-tailed phages with their hosts.

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1. Burns, C. M., Chan, H. L. & DuBow, M. S. In vitro maturation and encapsidation of the DNA of transposable Mu-like phage D108. *Proc. Natl Acad. Sci. USA* **87**, 6092–6096 (1990).
2. Stockley, P. G. et al. Bacteriophage MS2 genomic RNA encodes an assembly instruction manual for its capsid. *Bacteriophage* **6**, e1157666 (2016).
3. Ackermann, H.-W. & Prangishvili, D. Prokaryote viruses studied by electron microscopy. *Arch. Virol.* **157**, 1843–1849 (2012).
4. Hu, B., Margolin, W., Molineux, I. J. & Liu, J. Structural remodeling of bacteriophage T4 and host membranes during infection initiation. *Proc. Natl Acad. Sci. USA* **112**, E4919–E4928 (2015).
This study analyses cryo-electron microscopic images of phage T4 at different stages of infection to reveal that, contrary to common descriptions, most long tail fibres are folded back against the virion before infection.
5. Arnaud, C.-A. et al. Bacteriophage T5 tail tube structure suggests a trigger mechanism for *Siphoviridae* DNA ejection. *Nat. Commun.* **8**, 1953 (2017).
6. González-Garcīa, V. A. et al. Conformational changes leading to T7 DNA delivery upon interaction with the bacterial receptor. *J. Biol. Chem.* **290**, 10038–10044 (2015).
7. Taylor, N. M. I. et al. Structure of the T4 baseplate and its function in triggering sheath contraction. *Nature* **533**, 346–352 (2016).
This study presents the atomic structure of the phage T4 baseplate before and after attachment states, detailing the events leading to sheath contraction at the atomic level.
8. Keen, E. C. & Adhya, S. L. Review of phage therapy: current research and applications. *Clin. Infect. Dis.* **61**, 141–142 (2015).
9. Motlagh, A. M. et al. Insights of phage-host interaction in hypersaline ecosystem through metagenomics analyses. *Front. Microbiol.* **8**, 352 (2017).
10. Leiman, P. G. & Shneider, M. Contractile tail machines of bacteriophages. *Adv. Exp. Med. Biol.* **726**, 93–114 (2012).
11. Leiman, P. G. et al. Morphogenesis of the T4 tail and tail fibers. *Virol. J.* **7**, 355–355 (2010).
12. Kellenberger, E. et al. Functions and properties related to the tail fibers of bacteriophage T4. *Virology* **26**, 419–440 (1965).
13. Kostyuchenko, V. A. et al. The tail structure of bacteriophage T4 and its mechanism of contraction. *Nat. Struct. Mol. Biol.* **12**, 810 (2005).
14. Tētart, F., Repoila, F., Monod, C. & Krisch, H. M. Bacteriophage T4 host range is expanded by duplications of a small domain of the tail fiber adhesin. *J. Mol. Biol.* **258**, 726–731 (1996).
15. Ge, P. et al. Atomic structures of a bactericidal contractile nanotube in its pre- and postcontraction states. *Nat. Struct. Mol. Biol.* **22**, 377–382 (2015).
16. Büttner, C. R., Wu, Y., Maxwell, K. L. & Davidson, A. R. Baseplate assembly of phage Mu: defining the conserved core components of contractile-tailed phages and related bacterial systems. *Proc. Natl Acad. Sci. USA* **113**, 10174–10179 (2016).
This study defines the assembly pathway of the phage Mu baseplate and demonstrates that Mu-like, simple baseplates are conserved in

prophages and are the most likely evolutionary precursors of contractile, tail-derived bacterial apparatuses, such as the T6SS.
17. Nováček, J. et al. Structure and genome release of Twort-like *Myoviridae* phage with a double-layered baseplate. *Proc. Natl Acad. Sci. USA* **113**, 9351–9356 (2016).
18. Chibani-Chennoufi, S., Dillmann, M.-L., Marvin-Guy, L., Rami-Shojaei, S. & Brüssow, H. *Lactobacillus plantarum* bacteriophage LP65: a new member of the SPO1-like genus of the family *Myoviridae*. *J. Bacteriol.* **186**, 7069–7083 (2004).
19. Kutter, E. M. et al. Characterization of a Vil-like phage specific to *Escherichia coli* O157:H7. *Virol. J.* **8**, 430–430 (2011).
20. Arachchi, G. J. G. et al. Characteristics of three listeriaphages isolated from New Zealand seafood environments. *J. Appl. Microbiol.* **115**, 1427–1438 (2013).
21. Fokine, A. & Rossmann, M. G. Molecular architecture of tailed double-stranded DNA phages. *Bacteriophage* **4**, e28281 (2014).
22. Zivanovic, Y. et al. Insights into bacteriophage T5 structure from analysis of its morphogenesis genes and protein components. *J. Virol.* **88**, 1162–1174 (2014).
23. Böhm, J. et al. FhuA-mediated phage genome transfer into liposomes. *Curr. Biol.* **11**, 1168–1175 (2001).
24. Flayhan, A. et al. Crystal structure of pb9, the distal tail protein of bacteriophage T5: a conserved structural motif among all siphophages. *J. Virol.* **88**, 820–828 (2014).
25. Golomidova, A. et al. Branched lateral tail fiber organization in T5-like bacteriophages DT57C and DT571/2 is revealed by genetic and functional analysis. *Viruses* **8**, 26 (2016).
26. Scholl, D., Rogers, S., Adhya, S. & Merril, C. R. Bacteriophage K1-5 encodes two different tail fiber proteins, allowing it to infect and replicate on both K1 and K5 strains of *Escherichia coli*. *J. Virol.* **75**, 2509–2515 (2001).
27. Prokhorov, N. S. et al. Function of bacteriophage G7C esterase tailspike in host cell adsorption. *Mol. Microbiol.* **105**, 385–398 (2017).
28. Farenc, C. et al. Molecular insights on the recognition of a *Lactococcus lactis* cell wall pellicle by the phage 1358 receptor binding protein. *J. Virol.* **88**, 7005–7015 (2014).
29. Sciara, G. et al. Structure of lactococcal phage p2 baseplate and its mechanism of activation. *Proc. Natl Acad. Sci. USA* **107**, 6852–6857 (2010).
30. Bebeacua, C. et al. Structure, adsorption to host, and infection mechanism of virulent lactococcal phage p2. *J. Virol.* **87**, 12302–12312 (2013).
31. McCabe, O. et al. The targeted recognition of *Lactococcus lactis* phages to their polysaccharide receptors. *Mol. Microbiol.* **96**, 875–886 (2015).
32. Mahony, J., Randazzo, W., Neve, H., Settanni, L. & van Sinderen, D. Lactococcal 949 group phages recognize a carbohydrate receptor on the host cell surface. *Appl. Environ. Microbiol.* **81**, 3299–3305 (2015).
33. Cuervo, A. et al. Structural characterization of the bacteriophage T7 tail machinery. *J. Biol. Chem.* **288**, 26290–26299 (2013).
34. Steinbacher, S. et al. Phage P22 tailspike protein: crystal structure of the head-binding domain at 2.3 Å, fully refined structure of the endorhamnosidase at 1.56 Å resolution, and the molecular basis of O-antigen recognition and cleavage. *J. Mol. Biol.* **267**, 865–880 (1997).
35. Israel, V. A model for the adsorption of phage P22 to *Salmonella typhimurium*. *J. Gen. Virol.* **40**, 669–673 (1978).
36. Olia, A. S., Casjens, S. & Cingolani, G. Structure of phage P22 cell envelope-penetrating needle. *Nat. Struct. Mol. Biol.* **14**, 1221–1226 (2007).
37. Olia, A. S., Casjens, S. & Cingolani, G. Structural plasticity of the phage P22 tail needle gp26 probed with xenon gas. *Protein Sci.* **18**, 537–548 (2009).
38. Perez, G. L., Huynh, B., Slater, M. & Maloy, S. Transport of phage P22 DNA across the cytoplasmic membrane. *J. Bacteriol.* **191**, 135–140 (2009).
39. Tang, J. et al. DNA poised for release in bacteriophage ø29. *Structure* **16**, 935–943 (2008).
This study presents the first asymmetrical 3D reconstruction of a tailed double-stranded DNA phage at subnanometre resolution, providing important insights into the interactions of protein and DNA components during phage assembly.
40. Xiang, Y. et al. Crystallographic insights into the autocatalytic assembly mechanism of a bacteriophage tail spike. *Mol. Cell* **34**, 375–386 (2009).
41. Xu, J., Gui, M., Wang, D. & Xiang, Y. The bacteriophage ϕ29 tail possesses a pore-forming loop for cell membrane penetration. *Nature* **534**, 544–547 (2016).
This study demonstrates that prokaryotic and eukaryotic viruses use similar mechanisms to breach the cell membrane. As in some non-enveloped eukaryotic viruses, phage ϕ29 forms a pore with six hydrophobic loops that form a channel through the membrane and unblock the tail tube to enable DNA ejection.
42. Chibani-Chennoufi, S., Bruttin, A., Dillmann, M.-L. & Brüssow, H. Phage-host interaction: an ecological perspective. *J. Bacteriol.* **186**, 3677–3686 (2004).
43. Taylor, N. M. I., van Raaij, M. J. & Leiman, P. G. Contractile injection systems of bacteriophages and related systems. *Mol. Microbiol.* **108**, 6–15 (2018).
44. Andres, D. et al. Tailspike interactions with lipopolysaccharide effect DNA ejection from phage P22 particles in vitro. *J. Biol. Chem.* **285**, 36768–36775 (2010).
45. Müller, J. J. et al. An intersubunit active site between supercoiled parallel beta helices in the trimeric tailspike endorhamnosidase of *Shigella flexneri* phage Sf6. *Structure* **16**, 766–775 (2008).
46. Tang, L., Marion, W. R., Cingolani, G., Prevelige, P. E. & Johnson, J. E. Three-dimensional structure of the bacteriophage P22 tail machine. *EMBO J.* **24**, 2087–2095 (2005).
47. Seul, A. et al. Bacteriophage P22 tailspike: structure of the complete protein and function of the interdomain linker. *Acta Cryst. D* **70**, 1336–1345 (2014).
This study resolves the crystal structure of the phage P22 tail spike by making a single amino-acid exchange in the linker sequence between the particle binding domain and the receptor binding domain of the protein. It also suggests a role of the linker in signal transmission from the distal tip of the tail spike to the phage head, eventually leading to DNA ejection.
48. Barbirz, S. et al. Crystal structure of *Escherichia coli* phage HK620 tailspike: podoviral tailspike endoglycosidase modules are evolutionarily related. *Mol. Microbiol.* **69**, 303–316 (2008).
49. Dobbins, A. T. et al. Complete genomic sequence of the virulent *Salmonella* bacteriophage SP6. *J. Bacteriol.* **186**, 1933–1944 (2004).
50. Walter, M. et al. Structure of the receptor-binding protein of bacteriophage Det7: a podoviral tail spike in a myovirus. *J. Virol.* **82**, 2265–2273 (2008).
51. Olszak, T. et al. The O-specific polysaccharide lyase from the phage LKA1 tailspike reduces *Pseudomonas* virulence. *Sci. Rep.* **7**, 16302 (2017).
52. Pickard, D. et al. A conserved acetyl esterase domain targets diverse bacteriophages to the Vi capsular receptor of *Salmonella enterica* serovar Typhi. *J. Bacteriol.* **192**, 5746–5754 (2010).
53. Szczeklik, H. & Taylor, A. The presence of Vi-polysaccharide deacetylase in three morphologically different Vi-bacteriophages. *Biul. Inst. Med. Morsk. Gdansk.* **24**, 165–167 (1973).
54. King, J. & Laemmli, U. Polypeptides of the tail fibres of bacteriophage T4. *J. Mol. Biol.* **62**, 465–477 (1971).
55. Cerritelli, M. E., Wall, J. S., Simon, M. N., Conway, J. F. & Steven, A. C. Stoichiometry and domainal organization of the long tail-fiber of bacteriophage T4: a hinged viral adhesin. *J. Mol. Biol.* **260**, 767–780 (1996).
56. Hashemolhosseini, S., Stierhof, Y. D., Hindennach, I. & Henning, U. Characterization of the helper proteins for the assembly of tail fibers of coliphages T4 and lambda. *J. Bacteriol.* **178**, 6258–6265 (1996).
57. Bartual, S. G. et al. Structure of the bacteriophage T4 long tail fiber receptor-binding tip. *Proc. Natl Acad. Sci. USA* **107**, 20287–20292 (2010).
58. Washizaki, A., Yonesaki, T. & Otsuka, Y. Characterization of the interactions between *Escherichia coli* receptors, LPS and OmpC, and bacteriophage T4 long tail fibers. *MicrobiologyOpen* **5**, 1003–1015 (2016).
59. Hendrix, R. & Duda, R. Bacteriophage lambda PaPa: not the mother of all lambda phages. *Science* **258**, 1145–1148 (1992).
60. van Raaij, M. J., Schoehn, G., Burda, M. R. & Miller, S. Crystal structure of a heat and protease-stable part of the bacteriophage T4 short tail fibre. *J. Mol. Biol.* **314**, 1137–1146 (2001).
61. Mason, W. S. & Haselkorn, R. Product of T4 gene 12. *J. Mol. Biol.* **66**, 445–469 (1972).
62. Thomassen, E. et al. The structure of the receptor-binding domain of the bacteriophage T4 short tail fibre reveals a knitted trimeric metal-binding fold. *J. Mol. Biol.* **331**, 361–373 (2003).
63. Burda, M. R. & Miller, S. Folding of coliphage T4 short tail fiber in vitro. *Eur. J. Biochem.* **265**, 771–778 (1999).
64. Leiman, P. G., Shneider, M. M., Mesyanzhinov, V. V. & Rossmann, M. G. Evolution of bacteriophage tails: structure of T4 gene product 10. *J. Mol. Biol.* **358**, 912–921 (2006).
65. Garcia-Doval, C., Luque, D., Castón, J. R., Boulanger, P. & van Raaij, M. J. Crystallization of the C-terminal domain of the bacteriophage T5 L-shaped fibre. *Acta Cryst. F* **69**, 1363–1367 (2013).
66. Schulz, E. C. & Ficner, R. Knitting and snipping: chaperones in β-helix folding. *Curr. Opin. Struct. Biol.* **21**, 232–239 (2011).
67. Garcia-Doval, C. et al. Structure of the receptor-binding carboxy-terminal domain of the bacteriophage T5 L-shaped tail fibre with and without its intra-molecular chaperone. *Viruses* **7**, 6424–6440 (2015).
68. Spinelli, S., Veesler, D., Bebeacua, C. & Cambillau, C. Structures and host-adhesion mechanisms of lactococcal siphophages. *Front. Microbiol.* **5**, 3 (2014).
69. Garcia-Doval, C. & van Raaij, M. J. Structure of the receptor-binding carboxy-terminal domain of bacteriophage T7 tail fibers. *Proc. Natl Acad. Sci. USA* **109**, 9390–9395 (2012).
70. Veesler, D. & Cambillau, C. A. Common evolutionary origin for tailed-bacteriophage functional modules and bacterial machineries. *Microbiol. Mol. Biol. Rev.* **75**, 423–433 (2011).
71. Pires, D. P., Oliveira, H., Melo, L. D. R., Sillankorva, S. & Azeredo, J. Bacteriophage-encoded depolymerases: their diversity and biotechnological applications. *Appl. Microbiol. Biotechnol.* **100**, 2141–2151 (2016).
72. Kanamaru, S. et al. Structure of the cell-puncturing device of bacteriophage T4. *Nature* **415**, 553 (2002).
73. Nakagawa, H., Arisaka, F. & Ishii, S. Isolation and characterization of the bacteriophage T4 tail-associated lysozyme. *J. Virol.* **54**, 460–466 (1985).
74. Boulanger, P. et al. Phage T5 straight tail fiber is a multifunctional protein acting as a tape measure and carrying fusogenic and muralytic activities. *J. Biol. Chem.* **283**, 13556–13564 (2008).
75. Cumby, N., Reimer, K., Mngin-Lecreulx, D., Davidson, A. & Maxwell, K. The phage tail tape measure protein, an inner membrane protein and a periplasmid chaperone play connected roles in the genome injection process of *E. coli* phage HK97. *Mol. Microbiol.* **96**, 437–447 (2015).
76. Bhardwaj, A., Olia, A. S., Walker-Kopp, N. & Cingolani, G. Domain organization and polarity of tail needle GP26 in the portal vertex structure of bacteriophage P22. *J. Mol. Biol.* **371**, 374–387 (2007).
77. Xiang, Y. et al. Crystal and cryoEM structural studies of a cell wall degrading enzyme in the bacteriophage φ29 tail. *Proc. Natl Acad. Sci. USA* **105**, 9552–9557 (2008).
78. Ray, A. et al. Type VI secretion system MIX-effectors carry both antibacterial and anti-eukaryotic activities. *EMBO Rep.* **18**, 1978–1990 (2017).
79. Yang, G., Dowling, A. J., Gerike, U., ffrench-Constant, R. H. & Waterfield, N. R. *Photorhabdus* virulence cassettes confer injectable insecticidal activity against the wax moth. *J. Bacteriol.* **188**, 2254–2261 (2006).
80. Berkane, E. et al. Interaction of bacteriophage lambda with its cell surface receptor: an in vitro study of binding of the viral tail protein gpJ to LamB (maltoporin). *Biochemistry* **45**, 2708–2720 (2006).
81. Stockdale, S. R. et al. The lactococcal phages Tuc2009 and TP901-1 incorporate two alternate forms of their tail fiber into their virions for infection specialization. *J. Biol. Chem.* **288**, 5581–5590 (2013).
82. Stamereilers, C., LeBlanc, L., Yost, D., Amy, P. S. & Tsourkas, P. K. Comparative genomics of 9 novel *Paenibacillus larvae* bacteriophages. *Bacteriophage* **6**, e1220349 (2016).
83. Ceyssens, P. et al. Comparative analysis of the widespread and conserved PB1-like viruses infecting *Pseudomonas aeruginosa*. *Environ. Microbiol.* **11**, 2874–2883 (2009).
84. Garbe, J. et al. Characterization of JG024, a *Pseudomonas aeruginosa* PB1-like broad host range phage under simulated infection conditions. *BMC Microbiol.* **10**, 1–10 (2010).
85. Li, L. et al. Neutron crystallographic studies of T4 lysozyme at cryogenic temperature. *Protein Sci.* **26**, 2098–2104 (2017).

86. Oliveira, H. et al. Characterization and genomic analyses of two newly isolated *Morganella* phages define distant members among *Tevenvirinae* and *Autographivirinae* subfamilies. *Sci. Rep.* **7**, 46157 (2017).
87. Koraimann, G. Lytic transglycosylases in macromolecular transport systems of Gram-negative bacteria. *Cell. Mol. Life Sci.* **60**, 2371–2388 (2003).
88. Piuri, M. & Hatfull, G. F. A peptidoglycan hydrolase motif within the mycobacteriophage TM4 tape measure protein promotes efficient infection of stationary phase cells. *Mol. Microbiol.* **62**, 1569–1585 (2006).
89. Kanamaru, S., Ishiwata, Y., Suzuki, T., Rossmann, M. G. & Arisaka, F. Control of bacteriophage T4 tail lysozyme activity during the infection process. *J. Mol. Biol.* **346**, 1013–1020 (2005).
90. Glonti, T., Chanishvili, N. & Taylor, P. W. Bacteriophage-derived enzyme that depolymerizes the alginic acid capsule associated with cystic fibrosis isolates of *Pseudomonas aeruginosa*. *J. Appl. Microbiol.* **108**, 695–702 (2010).
91. Majkowska-Skrobek, G. et al. Capsule-targeting depolymerase, derived from *Klebsiella* KP36 phage, as a tool for the development of anti-virulent strategy. *Viruses* **8**, 324 (2016).
92. Hamdi, S. et al. Characterization of two polyvalent phages infecting Enterobacteriaceae. *Sci. Rep.* **7**, 40349 (2017).
93. Gutiérrez, D. et al. Role of the pre-neck appendage protein (Dpo7) from phage vB_SepiS-phiIPLA7 as an anti-biofilm agent in staphylococcal species. *Front. Microbiol.* **6**, 1315 (2015).
94. Rakhuba, D., Kolomiets, E., Dey, E. & Novik, G. Bacteriophage receptors, mechanisms of phage adsorption and penetration into host cell. *Pol. J. Microbiol.* **59**, 145–155 (2010).
95. Köhler, T., Donner, V. & van Delden, C. Lipopolysaccharide as shield and receptor for R-pyocin-mediated killing in *Pseudomonas aeruginosa*. *J. Bacteriol.* **192**, 1921–1928 (2010).
96. Kim, M., Kim, S., Park, B. & Ryu, S. Core lipopolysaccharide-specific phage SSU5 as an auxiliary component of a phage cocktail for *Salmonella* Biocontrol. *Appl. Environ. Microbiol.* **80**, 1026–1034 (2014).
97. Zhao, X. et al. Outer membrane proteins ail and OmpF of *Yersinia pestis* are involved in the adsorption of T7-related bacteriophage Yep-phi. *J. Virol.* **87**, 12260–12269 (2013).
98. Kim, M. & Ryu, S. Spontaneous and transient defence against bacteriophage by phase-variable glucosylation of O-antigen in *Salmonella enterica* serovar Typhimurium. *Mol. Microbiol.* **86**, 411–425 (2012).
99. Gehring, K., Charbit, A., Brissaud, E. & Hofnung, M. Bacteriophage lambda receptor site on the *Escherichia coli* K-12 LamB protein. *J. Bacteriol.* **169**, 2103–2106 (1987).
100. Ricci, V. & Piddock, L. J. V. Exploiting the role of TolC in pathogenicity: identification of a bacteriophage for eradication of *Salmonella* serovars from poultry. *Appl. Environ. Microbiol.* **76**, 1704–1706 (2010).
101. Breyton, C. et al. Assessing the conformational changes of pb5, the receptor-binding protein of phage T5, upon binding to its *Escherichia coli* receptor FhuA. *J. Biol. Chem.* **288**, 30763–30772 (2013).
102. Rabsch, W. et al. FepA- and TonB-dependent bacteriophage H8: receptor binding and genomic sequence. *J. Bacteriol.* **189**, 5658–5674 (2007).
103. Rakieten, M. & Rakieten, T. Relationship between staphylococci and bacilli belonging to the subtilis group as shown by bacteriophage adsorption. *J. Bacteriol.* **34**, 285–300 (1937).
104. Baptista, C., Santos, M. A. & São-José, C. Phage SPP1 reversible adsorption to *Bacillus subtilis* cell wall teichoic acids accelerates virus recognition of membrane receptor YueB. *J. Bacteriol.* **190**, 4989–4996 (2008).
105. Xia, G. et al. Wall teichoic acid-dependent adsorption of staphylococcal siphovirus and myovirus. *J. Bacteriol.* **193**, 4006–4009 (2011).
106. Li, X. et al. An essential role for the baseplate protein Gp45 in phage adsorption to *Staphylococcus aureus*. *Sci. Rep.* **6**, 26455 (2016).
107. Davison, S., Couture-Tosi, E., Candela, T., Mock, M. & Fouet, A. Identification of the *Bacillus anthracis* γ phage receptor. *J. Bacteriol.* **187**, 6742–6749 (2005).
108. São-José, C. et al. The ectodomain of the viral receptor YueB forms a fiber that triggers ejection of bacteriophage SPP1 DNA. *J. Biol. Chem.* **281**, 11464–11470 (2006).
109. Monteville, M. R., Ardestani, B. & Geller, B. L. Lactococcal bacteriophages require a host cell wall carbohydrate and a plasma membrane protein for adsorption and ejection of DNA. *Appl. Environ. Microbiol.* **60**, 3204–3211 (1994).
110. Tzipilevich, E., Habusha, M. & Ben-Yehuda, S. Acquisition of phage sensitivity by bacteria through exchange of phage receptors. *Cell* **168**, 186–199 (2017).
111. Guerrero-Ferreira, R. C. et al. Alternative mechanism for bacteriophage adsorption to the motile bacterium *Caulobacter crescentus*. *Proc. Natl Acad. Sci. USA* **108**, 9963–9968 (2011).
This study demonstrates that some phages use a filament on the phage head to interact with the bacterial flagellum and thus concentrate phage particles around the receptor on the cell surface; the study suggests that similar mechanisms apply to other phages and hosts.
112. Choi, Y., Shin, H., Lee, J.-H. & Ryu, S. Identification and characterization of a novel flagellum-dependent *Salmonella*-infecting bacteriophage, iEPS5. *Appl. Environ. Microbiol.* **79**, 4829–4837 (2013).
113. Raimondo, L. M., Lundh, N. P. & Martinez, R. J. Primary adsorption site of phage PBS1: the flagellum of *Bacillus subtilis*. *J. Virol.* **2**, 256–264 (1968).
114. Lovett, P. S. PBP1: a flagella specific bacteriophage mediating transduction in *Bacillus pumilus*. *Virology* **47**, 743–752 (1972).
115. Evans, T. J. et al. Characterization of a broad-host-range flagellum-dependent phage that mediates high-efficiency generalized transduction in, and between, *Serratia* and *Pantoea*. *Microbiology* **156**, 240–247 (2010).
116. Evans, T. J., Trauner, A., Komitopoulou, E. & Salmond, G. P. C. Exploitation of a new flagellatropic phage of *Erwinia* for positive selection of bacterial mutants attenuated in plant virulence: towards phage therapy. *J. Appl. Microbiol.* **108**, 676–685 (2009).
117. Pate, J. L., Petzold, S. J. & Umbreit, T. H. Two flagellotropic phages and one pilus-specific phage active against *Asticcacaulis biprosthecum*. *Virology* **94**, 24–37 (1979).
118. Zhang, H., Li, L., Zhao, Z., Peng, D. & Zhou, X. Polar flagella rotation in *Vibrio parahaemolyticus* confers resistance to bacteriophage infection. *Sci. Rep.* **6**, 26147 (2016).
119. Chibeu, A. et al. The adsorption of *Pseudomonas aeruginosa* bacteriophage φKMV is dependent on expression regulation of type IV pili genes. *FEMS Microbiol. Lett.* **296**, 210–218 (2009).
120. Bae, H.-W. & Cho, Y.-H. Complete genome sequence of *Pseudomonas aeruginosa* podophage MPK7, which requires type IV pili for infection. *Genome Announc.* **1**, e00744–00713 (2013).
121. Costa & Tiago, R. et al. Structure of the bacterial sex F pilus reveals an assembly of a stoichiometric protein-phospholipid complex. *Cell* **166**, 1436–1444 (2016).
122. Frost, L. & Paranchych, W. DNA sequence analysis of point mutations in traA, the F pilin gene, reveal two domains involved in F-specific bacteriophage attachment. *Mol. Gen. Genet.* **213**, 134–139 (1988).
123. Harvey, H. et al. *Pseudomonas aeruginosa* defends against phages through type IV pilus glycosylation. *Nat. Microbiol.* **3**, 47–52 (2018).
124. Hsu, C.-R., Lin, T.-L., Pan, Y.-J., Hsieh, P.-F. & Wang, J.-T. Isolation of a bacteriophage specific for a new capsular type of *Klebsiella pneumoniae* and characterization of its polysaccharide depolymerase. *PLOS ONE* **8**, e70092 (2013).
125. Scholl, D., Adhya, S. & Merril, C. *Escherichia coli* K1's capsule is a barrier to bacteriophage T7. *Appl. Environ. Microbiol.* **71**, 4872–4874 (2005).
126. Oliveira, H. et al. Ability of phages to infect *Acinetobacter calcoaceticus-Acinetobacter baumannii* complex species through acquisition of different pectate lyase depolymerase domains. *Environ. Microbiol.* **19**, 5060–5077 (2017).
127. Ozaki, T., Abe, N., Kimura, K., Suzuki, A. & Kaneko, J. Genomic analysis of *Bacillus subtilis* lytic bacteriophage φNIT1 capable of obstructing natto fermentation carrying genes for the capsule-lytic soluble enzymes poly-γ-glutamate hydrolase and levanase. *Biosci. Biotechnol. Biochem.* **81**, 135–146 (2017).
128. Lee, K. K. & Gui, L. Dissecting virus infectious cycles by cryo-electron microscopy. *PLOS Pathog.* **12**, e1005625 (2016).
This study elucidates the mechanism of genome ejection of a Twort-like myophage: tail sheath contraction induces conformational changes to release the DNA from the connector complex; the DNA enters the neck; and further structural change in the tail tube enables genome release.
129. Orlov, I. & Klaholz, B. in *Europ. Microsc. Congress 2016 Proc.* 27–28 (2016).
130. Doerr, A. Single-particle cryo-electron microscopy. *Nat. Methods* **13**, 23–23 (2016).
131. Kauffman, K. M. et al. A major lineage of non-tailed dsDNA viruses as unrecognized killers of marine bacteria. *Nature* **554**, 118 (2018).
132. Schooley, R. T. et al. Development and use of personalized bacteriophage-based therapeutic cocktails to treat a patient with a disseminated resistant *Acinetobacter baumannii* infection. *Antimicrob. Agents Chemother.* **61**, e00954–e00917 (2017).
133. Zhvania, P., Hoyle, N. S., Nadareishvili, L., Nizharadze, D. & Kutateladze, M. Phage therapy in a 16-year-old boy with Netherton syndrome. *Front. Med.* **4**, 94 (2017).
134. Abedon, S. in *Phage Therapy: Current Research and Applications* Ch. 3 (eds Borysowski, J., Międzybrodzki, R. & Górski, A.) (Caister Academic Press, 2014).
135. Cui, L. & Bikard, D. Consequences of Cas9 cleavage in the chromosome of Escherichia coli. *Nucleic Acids Res.* **44**, 4243–4251 (2016).
136. Nobrega, F. L. et al. Genetically manipulated phages with improved pH resistance for oral administration in veterinary medicine. *Sci. Rep.* **6**, 39235 (2016).
137. Das, M., Bhowmick, T. S., Ahern, S. J., Young, R. & Gonzalez, C. F. Control of Pierce's disease by phage. *PLOS ONE* **10**, e0128902 (2015).
138. Wang, Y. et al. Bacteriophage therapy for the control of *Vibrio harveyi* in greenlip abalone (*Haliotis laevigata*). *Aquaculture* **473**, 251–258 (2017).
139. Bao, H. et al. Bio-control of *Salmonella enteritidis* in foods using bacteriophages. *Viruses* **7**, 4836–4853 (2015).
140. Mortari, A., Adami, A. & Lorenzelli, L. An unconventional approach to impedance microbiology: detection of culture media conductivity variations due to bacteriophage generated lyses of host bacteria. *Biosens. Bioelectron.* **67**, 615–620 (2015).
141. Chen, J., Alcaine, S. D., Jiang, Z., Rotello, V. M. & Nugen, S. R. Detection of *Escherichia coli* in drinking water using T7 bacteriophage-conjugated magnetic probe. *Anal. Chem.* **87**, 8977–8984 (2015).
142. Sergueev, K. V., Filippov, A. A. & Nikolich, M. P. Highly sensitive bacteriophage-based detection of *Brucella abortus* in mixed culture and spiked blood. *Viruses* **9**, 144 (2017).
143. McNerney, R., Kambashi, B. S., Kinkese, J., Tembwe, R. & Godfrey-Faussett, P. Development of a bacteriophage phage replication assay for diagnosis of pulmonary tuberculosis. *J. Clin. Microbiol.* **42**, 2115–2120 (2004).
144. Vinay, M. et al. Phage-based fluorescent biosensor prototypes to specifically detect enteric bacteria such as *E. coli* and *Salmonella enterica* Typhimurium. *PLOS ONE* **10**, e0131466 (2015).
145. Arya, S. K. et al. Chemically immobilized T4-bacteriophage for specific *Escherichia coli* detection using surface plasmon resonance. *Analyst* **136**, 486–492 (2011).
146. Srivastava, S. K. et al. Highly sensitive and specific detection of *E. coli* by a SERS nanobiosensor chip utilizing metallic nanosculptured thin films. *Analyst* **140**, 3201–3209 (2015).
147. Moghtader, F., Congur, G., Zareie, H. M., Erdem, A. & Piskin, E. Impedimetric detection of pathogenic bacteria with bacteriophages using gold nanorod deposited graphite electrodes. *RSC Adv.* **6**, 97832–97839 (2016).
148. Janczuk, M. et al. Bacteriophage-based bioconjugates as a flow cytometry probe for fast bacteria detection. *Bioconjug. Chem.* **28**, 419–425 (2017).
149. Wang, Z., Wang, D., Kinchla, A. J., Sela, D. A. & Nugen, S. R. Rapid screening of waterborne pathogens using phage-mediated separation coupled with real-time PCR detection. *Anal. Bioanal. Chem.* **408**, 4169–4178 (2016).
150. Zhang, Y., Yan, C., Yang, H., Yu, J. & Wei, H. Rapid and selective detection of *E. coli* O157:H7 combining phagomagnetic separation with enzymatic colorimetry. *Food Chem.* **234**, 332–338 (2017).
151. Tay, L.-L. et al. Silica encapsulated SERS nanoprobe conjugated to the bacteriophage tailspike protein for targeted detection of *Salmonella*. *Chem. Comm.* **48**, 1024–1026 (2012).
152. Schmidt, A., Rabsch, W., Broeker, N. K. & Barbirz, S. Bacteriophage tailspike protein based assay to monitor phase variable glucosylations in *Salmonella* O-antigens. *BMC Microbiol.* **16**, 207 (2016).

153. Pan, Y.-J. et al. Identification of capsular types in carbapenem-resistant *Klebsiella pneumoniae* strains by wzc sequencing and implications for capsule depolymerase treatment. *Antimicrob. Agents Chemother.* **59**, 1038–1047 (2015).
154. Lin, T.-L. et al. Isolation of a bacteriophage and its depolymerase specific for K1 capsule of *Klebsiella pneumoniae*: implication in typing and treatment. *J. Infect. Dis.* **210**, 1734–1744 (2014).
155. Hsieh, P.-F., Lin, H.-H., Lin, T.-L., Chen, Y.-Y. & Wang, J.-T. Two T7-like bacteriophages, K5-2 and K5-4, each encodes two capsule depolymerases: Isolation and functional characterization. *Sci. Rep.* **7**, 4624 (2017).
156. Nakayama, K. et al. The R-type pyocin of *Pseudomonas aeruginosa* is related to P2 phage, and the F-type is related to lambda phage. *Mol. Microbiol.* **38**, 213–231 (2000).
157. Scholl, D. Phage tail-like bacteriocins. *Annu. Rev. Virol.* **4**, 453–467 (2017).
This review provides a comprehensive overview of the function, structure and engineering of antibacterial tailocins that are released by cell lysis.
158. Uratani, Y. & Hoshino, T. Pyocin R1 inhibits active transport in *Pseudomonas aeruginosa* and depolarizes membrane potential. *J. Bacteriol.* **157**, 632–636 (1984).
159. Sarris, P. F., Ladoukakis, E. D., Panopoulos, N. J. & Scoulica, E. V. A phage tail-derived element with wide distribution among both prokaryotic domains: a comparative genomic and phylogenetic study. *Genome Biol. Evol.* **6**, 1739–1747 (2014).
160. Hurst, M. R., Beard, S. S., Jackson, T. A. & Jones, S. M. Isolation and characterization of the *Serratia entomophila* antifeeding prophage. *FEMS Microbiol. Lett.* **270**, 42–48 (2007).
161. Shikuma, N. J. et al. Marine tubeworm metamorphosis induced by arrays of bacterial phage tail-like structures. *Science* **343**, 529–533 (2014).
162. Leiman, P. G. et al. Type VI secretion apparatus and phage tail-associated protein complexes share a common evolutionary origin. *Proc. Natl Acad. Sci. USA* **106**, 4154–4159 (2009).
163. Boyer, F., Fichant, G., Berthod, J., Vandenbrouck, Y. & Attree, I. Dissecting the bacterial type VI secretion system by a genome wide in silico analysis: what can be learned from available microbial genomic resources? *BMC Genomics* **10**, 104 (2009).
164. Chang, Y. W., Rettberg, L. A., Ortega, D. R. & Jensen, G. J. In vivo structures of an intact type VI secretion system revealed by electron cryotomography. *EMBO Rep.* **18**, 1090–1099 (2017).
165. Gallique, M., Bouteiller, M. & Merieau, A. The type VI secretion system: a dynamic system for bacterial communication? *Front. Microbiol.* **8**, 1454 (2017).
166. Kohler, T., Donner, V. & van Delden, C. Lipopolysaccharide as shield and receptor for R-pyocin-mediated killing in *Pseudomonas aeruginosa*. *J. Bacteriol.* **192**, 1921–1928 (2010).
167. Williams, S. R., Gebhart, D., Martin, D. W. & Scholl, D. Retargeting R-type pyocins to generate novel bactericidal protein complexes. *Appl. Environ. Microbiol.* **74**, 3868–3876 (2008).
This innovative paper describes how the host range of R-type pyocins can be reprogrammed by replacing parts of the tail fibres between phages with different host ranges.
168. Gebhart, D. et al. A modified R-type bacteriocin specifically targeting Clostridium difficile prevents colonization of mice without affecting gut microbiota diversity. *mBio* **6**, e02368 (2015).
169. Steinbacher, S. et al. Crystal structure of P22 tailspike protein: interdigitated subunits in a thermostable trimer. *Science* **265**, 383–386 (1994).
170. Heller, K. & Braun, V. Accelerated adsorption of bacteriophage T5 to *Escherichia coli* F, resulting from reversible tail fiber-lipopolysaccharide binding. *J. Bacteriol.* **139**, 32–38 (1979).
171. Wang, J., Hofnung, M. & Charbit, A. The c-terminal portion of the tail fiber protein of bacteriophage lambda is responsible for binding to LamB, its receptor at the surface of *Escherichia coli* K-12. *J. Bacteriol.* **182**, 508–512 (2000).
172. German, G. J. & Misra, R. The TolC protein of *Escherichia coli* serves as a cell-surface receptor for the newly characterized TLS bacteriophage1. *J. Mol. Biol.* **308**, 579–585 (2001).
173. Schade, S. Z., Adler, J. & Ris, H. How bacteriophage χ attacks motile bacteria. *J. Virol.* **1**, 599–609 (1967).
174. Budzik, J. M., Rosche, W. A., Rietsch, A. & O'Toole, G. A. Isolation and characterization of a generalized transducing phage for *Pseudomonas aeruginosa* strains PAO1 and PA14. *J. Bacteriol.* **186**, 3270–3273 (2004).
175. Munsch-Alatossava, P. & Alatossava, T. The extracellular phage-host interactions involved in the bacteriophage LL-H infection of *Lactobacillus delbrueckii* ssp. *lactis* ATCC 15808. *Front. Microbiol.* **4**, 408 (2013).
176. Andres, D. et al. Tail morphology controls DNA release in two *Salmonella* phages with one lipopolysaccharide receptor recognition system. *Mol. Microbiol.* **83**, 1244–1253 (2012).
177. Vinga, I. et al. Role of bacteriophage SPP1 tail spike protein gp21 on host cell receptor binding and trigger of phage DNA ejection. *Mol. Microbiol.* **83**, 289–303 (2012).
178. Bradbeer, C., Woodrow, M. L. & Khalifah, L. I. Transport of vitamin B_{12} in *Escherichia coli*: common receptor system for vitamin B_{12} and bacteriophage BF23 on the outer membrane of the cell envelope. *J. Bacteriol.* **125**, 1032–1039 (1976).
179. Heller, K. J. & Schwarz, H. Irreversible binding to the receptor of bacteriophages T5 and BF23 does not occur with the tip of the tail. *J. Bacteriol.* **162**, 621–625 (1985).
180. Sandulache, R., Prehm, P., Expert, D., Toussaint, A. & Kamp, D. The cell wall receptor for bacteriophage Mu G(–) in *Erwinia* and *Escherichia coli* C. *FEMS Microbiol. Lett.* **28**, 307–310 (1985).
181. Yoichi, M., Abe, M., Miyanaga, K., Unno, H. & Tanji, Y. Alteration of tail fiber protein gp38 enables T2 phage to infect *Escherichia coli* O157:H7. *J. Biotechnol.* **115**, 101–107 (2005).
182. Lee, J.-H., Shin, H., Kim, H. & Ryu, S. Complete genome sequence of *Salmonella* bacteriophage SPN3US. *J. Virol.* **85**, 13470–13471 (2011).
183. Habann, M. et al. *Listeria* phage A511, a model for the contractile tail machineries of SPO1-related bacteriophages. *Mol. Microbiol.* **92**, 84–99 (2014).
184. Matilla, M. A. & Salmond, G. P. C. Bacteriophage ϕMAM1, a Viunalikevirus, is a broad-host-range, high-efficiency generalized transducer that infects environmental and clinical isolates of the enterobacterial genera *Serratia* and *Kluyvera*. *Appl. Environ. Microbiol.* **80**, 6446–6457 (2014).
185. Prehm, P., Jann, B., Jann, K., Schmidt, G. & Stirm, S. On a bacteriophage T3 and T4 receptor region within the cell wall lipopolysaccharide of *Escherichia coli* B. *J. Mol. Biol.* **101**, 277–281 (1976).
186. Parent, K. N. et al. OmpA and OmpC are critical host factors for bacteriophage Sf6 entry in *Shigella*. *Mol. Microbiol.* **92**, 47–60 (2014).

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