Phage fibers and spikes: a nanoscale Swiss army knife for host infection

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Phage fibers and spikes: a nanoscale Swiss army knife for host infection

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Bacteriophages are being rediscovered as potent agents for medical and industrial applications. However, finding a suitable phage relies on numerous factors, including host specificity, burst size, and infection cycle. The host range of a phage is, besides phage defense systems, initially determined by the recognition and attachment of receptor-binding proteins (RBPs) to the target receptors of susceptible bacteria. RBPs include tail (or occasionally head) fibers and tailspikes. Owing to the potential flexibility and heterogeneity of these structures, they are often overlooked during structural studies. Recent advances in cryo-electron microscopy studies and computational approaches have begun to unravel their structural and fundamental mechanisms during phage infection. In this review, we discuss the current state of research on different phage tail and head fibers, spike models, and molecular mechanisms. These details may facilitate the manipulation of phage-host specificity, which in turn will have important implications for science and society.

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Introduction

Viruses that infect bacteria, called bacteriophages or phages for short, are ubiquitous in nature. They are a major driving force of bacterial evolution and the structure of environmental bacterial communities. Furthermore, the ability of phages to kill pathogenic bacteria has been successfully utilized for a variety of initiatives, including disease treatment caused by antimicrobial-resistant pathogens [1], microbial source-tracking and fecal indicators [2], and biocontrol agents used during food production [1,3].

A common feature of all phages is that their genetic material (single- or double-stranded DNA or RNA) is enclosed by a protein coat called a capsid. In addition, the vast majority of phages also possess a phage tail [4]. The highly ordered structures of phage capsids and tails have made them exceptionally suitable targets for structural studies [5–8], and these studies have provided enormous impact on the phage research field.

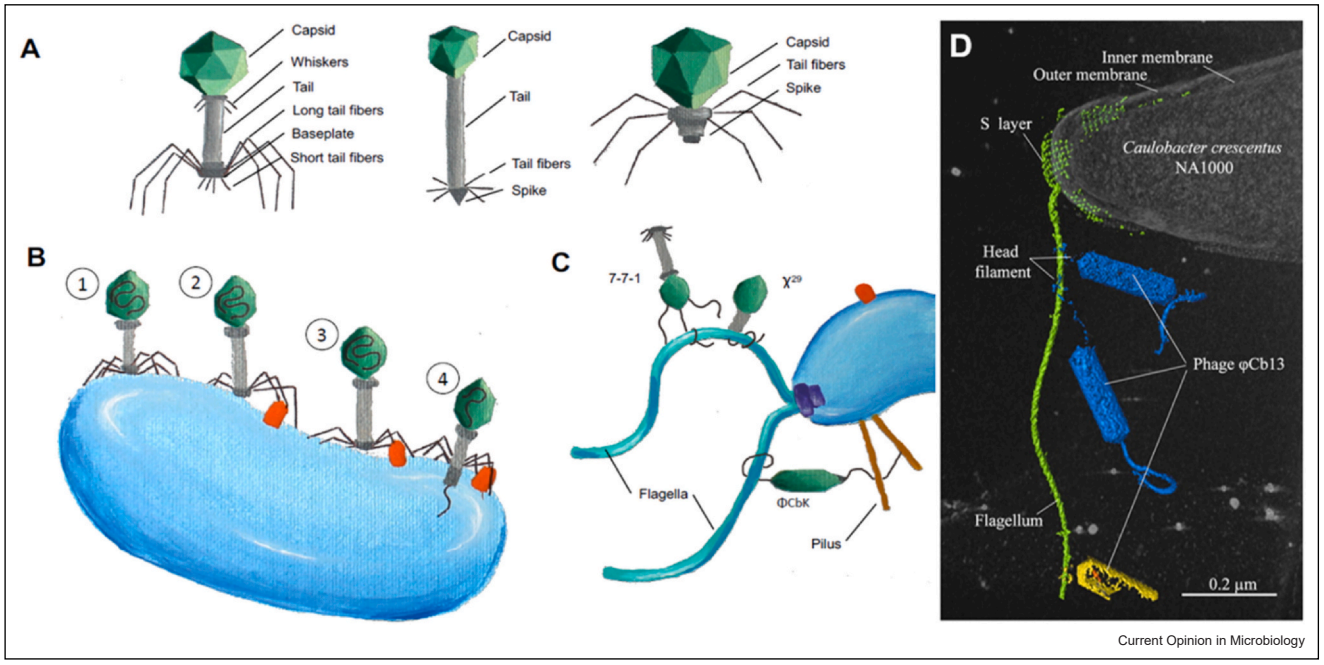
Phages recognize a suitable host with receptor-binding proteins (RBPs), such as tail or head fibers, tailspikes, or the central tailspike [5] that play a crucial role in the viral replication cycle by facilitating the attachment and/or entry of the viral genome into the host cell. In this review, we will discuss important structural and functional aspects of these fibers and spikes, which are structurally less well-characterized.

The structure of phage tail fibers and tailspikes is often difficult to determine by conventional methods, such as X-ray crystallography or nuclear magnetic resonance spectroscopy, because of their flexibility, heterogeneity, and low abundance in the virion. Therefore, they have been difficult to study from a structural perspective until the advent of cryo-electron microscopy (cryo-EM). This method has enabled the structural elucidation of some stable, ordered tail fibers and tailspikes at high resolution [9,10]. However, flexible or thin RBPs within these samples are often averaged out during image processing. A detailed understanding of phage RBPs will enable us to generate a complete molecular model for viral attachment. In turn, these insights may be used to genetically engineer altered phage fibers or spikes with improved efficiency for medical and industrial applications.

Tail fibers and tailspikes and their interaction with host receptors

Tailed phages can be classified into three different morphotypes: myoviruses, siphoviruses, and podoviruses (Figure 1a) [11,12]. Tail fibers are composed of repetitive protein subunits that form a rod-like structure with a

Figure 1



Morphology and infection mechanisms of phages. **(a)** The general morphology of the three phage morphotypes. Myoviruses (left) possess a capsid that contains DNA, a tubular tail with a contractile sheath, and a baseplate with tail fibers and/or tailspikes. Siphoviruses (center) possess a capsid that contains DNA, a noncontractile tubular tail, terminal tail fibers, and a spike protein that allows for adsorption into the host cell. Podoviruses (right) possess a capsid that contains DNA, a short noncontractile tail that consists of stacked protein disks with tail fibers, and a tailspike that allows for adsorption into the host cell. **(b)** The myovirus infection model of T4 occurs in four general steps. First, the phage uses its LTFs to scan the surface of the cell for its receptor. Second, the phage identifies its target receptor (orange square) with the STFs. Third, the phage will attach to the cell and undergo structural changes that allow it to penetrate the cell. Last, the phage will inject its DNA into the target cell by extending its contractile sheath. **(c)** Examples of flagellotropic phages. Using either head or tail fibers, phages can attach to the flagella of cells (teal) to infect their hosts. In some cases, the phages have a secondary receptor such as pili (yellow) or cell surface proteins (orange). **(d)** The segmentation of ϕCb13-infected NA1000 *C. crescentus* cells [19]. Notice the head filaments that extend from the phage and wrap around the flagellum. Image is used with permission.

globular domain at the distal end, which is used for recognition of the host cell. The structure, number of subunits, and location of the tail fibers differ among the phage classes [5]. In contrast to tailspikes, the tail fibers do not carry enzymes to degrade the cell wall or facilitate DNA ejection [5]. Myoviruses possess long, contractile tails covered in a tail sheath with a baseplate that links to tailspikes and long tail fibers (LTFs), with or without accessory proteins, and/or short tail fibers (STFs). Siphoviruses display long, noncontractile tails with a baseplate that links to LTFs and STFs, and a central tail fiber or spike that can bind to various host receptors. Podoviruses exhibit short, noncontractile tails with tail fibers or spikes and lack a baseplate. Nevertheless, possessing a large diversity of RBPs does not always correlate to a broad host range. Even phages with a single tail fiber or tailspike can have a broad host range when their target receptor is widespread [13,14].

The RBPs interact with cell wall receptors located on the surface of host cells. These target receptors can vary across bacterial species and the cell envelope structure. The receptors are generally classified as either primary or secondary receptors [15], although some phages recognize a single receptor [16]. Primary receptors are located on structures that protrude from the bacterial cell surface such as pili, flagella, or capsules, and can enhance or modulate phage adsorption and infection. Secondary receptors are typically located on the outermost layer of the bacterial cell envelope, such as lipopolysaccharides (LPS) in Gram-negative bacteria or teichoic acids in Gram-positive bacteria [17]. The initial binding of tail fibers or spikes to receptors is reversible and weak, allowing the phage to scan and select the appropriate host cells. The RBPs recognize and bind to receptors (Figure 1b), which induces conformational changes within the phage that in turn results in irreversible and strong binding and the activation of the subsequent infection steps [14]. Among other factors such as defense systems, the specificity and affinity of the RBP-receptor interaction determine the host range and infection efficiency of phages. The wide variety of bacterial cell wall receptors exemplifies the evolutionary 'arms race' that occurs between phages and bacteria. Phages have evolved to

recognize and bind to different receptors depending on their host specificity and environmental conditions. Bacteria have evolved to modify or mask their surface receptors to resist phage infection [18].

Long and short tail fibers

LTFs and STFs play various roles in phage attachment and genome injection. There are a few established infection models that demonstrate these mechanistic differences. Many myovirus phages [20], such as T4 [10], have two types of tail fibers, LTFs and STFs that are primarily responsible for binding to cell surface receptors [21,22]. Aside from T4, there are many other phages that have two kinds of tail fibers. For example, bacteriophage RB49, and its relatives Cognac and Whisky, use O-antigens as one of their alternative primary receptors, which is recognized by the LTF RBP gp38 [22]. Subsequently, the short tailspikes of these phages penetrate the O-antigen layer and trigger viral DNA injection. Phage SU10 [23] utilizes a unique nozzle to deliver its genome into the bacterial cytoplasm. Specifically, the binding of LTFs to the target receptors in the outer bacterial membrane induces straightening of the nozzle proteins and rotation of STFs. After the rearrangement, the nozzle proteins and STFs alternate to form a new nozzle structure that extends the tail [24].

Flagellotropic bacteriophages and head fibers

Flagellotropic bacteriophages are phages that attach to the flagellum of their motile host in the initial phase of infection (Figure 1c). Flagella are important bacterial appendages that enable swimming and swarming motilities and contribute to virulence in many pathogenic bacteria. Phages that recognize flagella can induce an interesting evolutionary trade-off with their host bacteria. This is comparable to the host-phage evolution of capsule-targeting phages that can trigger the evolution of bacterial strains that lack a capsule but are less virulent, and are therefore eliminated by the host immune system [25]. Bacteria can also suppress or eliminate the ability to move the flagella and escape from flagellotropic phages, but the pathogen may become less virulent [26]. Therefore, understanding the attachment mechanisms of flagellotropic phages, their molecular basis of infection, and their potential practical applications is of great importance.

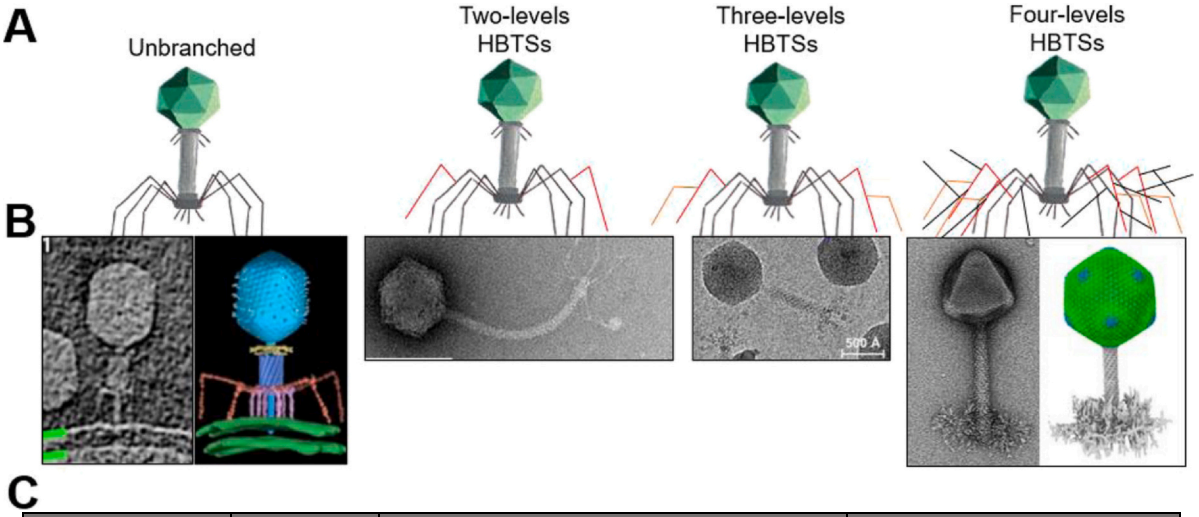
Several studies have revealed the diversity and complexity of flagellotropic phages and their infection process [27,28,24–32]. For example, bacteriophage F341 [29] can still infect *Campylobacter jejuni* lacking a capsule, but requires rotating flagella for successful infection. Paralyzed flagella of the Gram-positive bacteria *Bacillus subtilis* can still be infected by phage PBS1 with its multiple corkscrew-shaped tail fibers, while phage χ needs functional *B. subtilis* flagella [30,31]. Bacteriophage φχ [32] infects multiple genera of *Enterobacterales* using its single LTF that curls into a coil near the distal tip of the phage fiber. Using this fiber, the phage binds to the host flagellum and then uses the efflux system AcrABZ-TolC to transfer phage DNA into the host cell [33,34]. Phage 7–7-1 [35,36] infects *Agrobacterium sp. H13-3* using its head fibers and the rotation of the flagellum to reach the cell surface, where it interacts with LPS as a secondary receptor [37]. Some phages, such as bacteriohhage phiCBPK (ΦCbK) [38], infect *Caulobacter crescentus* using its single long head fiber and the rotation of the flagellum to reach the cell surface (Figure 1d), where the tail fibers interact with the pilus portal protein [39]. *Caulobacter* phages ΦCb13 and Φ6 are closely related to ΦCbK and likely also infect *C. crescentus* via the flagellum [19,38]. So far, ΦCbK and its direct family are unique in using a single long head filament to capture and attach to the flagellum. So far, these interactions have only been studied in phage-host pairs. However, different bacterial species can possess flagella that have similar flagellin proteins and surface grooves. These similarities may cause flagellotropic phages to ride a flagellum to the cell surface but fail in proceeding with infection as it has attached to a nonhost bacterium.

Hyperbranching tailspikes and fibers

The structure of tail fibers and tailspikes can be branched or unbranched. The branching RBPs can form complex structures and are referred to as hyperbranching tailspikes (HBTSs). These HBTSs allow for a greater degree of specificity in recognizing and binding to target cells. The branching structure is thought to increase the surface area, thereby increasing the likelihood of encountering and binding to the appropriate receptor [5,14]. In addition, different types of tailspikes and tail fibers formed by a variety of RBPs increase the variety of receptors the phage can bind to.

Tailspikes typically share structural similarities. The N-terminus of the tailspikes plays a structural role in attachment to the phage tails, or can serve as a docking site for other tailspikes or fibers [40]. The C-terminus contains the enzymatic depolymerase domain that defines capsule serotype and LPS–O-antigen specificity. The C-terminus may also include additional chaperones or carbohydrate-binding domains [42–47]. Based on recent studies [40,41,60], the structural model of HBTSs is postulated to resemble a multilevel branch bifurcation, similar to a branched tree (Figure 2). The putative tailspike proteins can be divided into levels, based on the length of the N-terminal structural segment [38]. In this model, the primary-level tailspike (the tree trunk) contains the largest N-terminal structural domain that enables it to attach the full hyperbranched system to the baseplate and serves as a docking site for secondary

Figure 2



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	Phage	Host	Reference
Unbranched	**T4**	*E. coli*	Leiman, P. G. *et al.*[10]
	T7	*E. coli*	You, L. *et al.*[44]
Two-levels HBTSs	**DT57C**	*E. coli* O22, O81	Golomidova, A. K. *et al.*[45]
	DT571/2	*E. coli* O87, O81	
	KP32	*Klepsiella pneumoniae* K3, K21	Latka, A. *et al.*[40]
	G7C	*E. coli* 4s	Prokhorov, N. S. *et al.*[46]
	EP335	*E. coli* serotype O157, O26	Witte, S. *et al.*[47]
	K1-5	*E. coli* serotype K1, K5	Leiman, P. G. *et al.*[48]
	AKFV33	*E. coli* O157:H7	Niu, Y. D. *et al.*[49]
	phiEco32	*E. coli* 55	Savalia, D. *et al.*[50]
Three-levels HBTSs	**Phi92**	*E. coli* K1, K-12, K92, K-235, EV5, BL21-Gold, Nissle 1917 & nineteen *Salmonella serovars*	Schwarzer, D. *et al.*[51]
Four-levels HBTSs	**Vi phage I**	*Salmonella enterica serovar* Typhi	Adriaenssens, E. M. *et al.*[52]
	φKp24	*Klebsiella pneumoniae* K2, K13, K19, K25, K35, K46, K61, K64, K81	Ouyang, R. *et al.*[41]
	CBA120	*E. coli* O77, O78, O157 & *Salmonella enterica serovar* Minnesota	Plattner, M. *et al.*[53]
	EP75	*E. coli* O18A, O157 & *Salmonella* O4, O7, O9	Witte, S. *et al.*[47]

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Phage tail fibers and spikes vary in structure. While some RBPs are unbranched and assemble into a singular protein filament, other phages possess RBPs that are branched. **(a)** In primary branched phages, RBPs extend from other RBPs that are directly attached to the phage baseplate (red). In secondary branched phages, RBPs extend from the primary branched RBPs (orange). In four-level branched phages, RBPs are highly heterogeneous and possess a multilevel branched structure (black). **(b)** From left to right, a tomogram and 3D rendered image of the unbranched phage T4 [54]. Negative staining of the two-level HBTS phage DT571/2 [45]. Cryo-EM image of phage Phi92 to represent three-level HBTSs [49]. Negative staining and 3D reconstruction of four-level HBTS phage φKp24 [41,55]. All images are used with permission. **(c)** HBTS-phage table: Unbranched phages: T4 [10], T7 [44]; two-level HBTS phages: DT57C, DT571/2 [45], KP32 [40], G7C [46], EP335 [47], K1–5 [48], AKFV33 [49], and phiEco32 [50]; three-level HBTS phage: Phi92 [51]; four-level HBTS phages: Vi phage I [52], ϕKp24 [41], CBA120 [53], and EP75 [47].

RBPs via specific domains (such as T4gp10-like domains). Secondary-level RBPs can comprise additional docking sites for tertiary-level RBPs. Fourth-level RBPs can attach to the third-level RBPs or attach to the primary-level tailspike. Consequently, the different groups of RBPs correspond to more peripheral positions in the tail fiber or tailspike arrangement. For example, phage ϕKp24 has 14 putative depolymerases, but is classified as a four-level HBTS phage, based on the length of the N-terminal structural domains [38]. Recent research has focused on understanding the function of HBTSs in different phages, as well as their potential applications in biotechnology and medicine [42,43].

The characterization of some phages with HBTSs has been challenging due to limited investigations into their structural and morphological properties. In the future, it would be valuable to identify additional multilevel HBTS phages, since they are particularly understudied and the quaternary assembly of their RBPs remains largely unknown. As an illustration, phage ΦK64-1 [56] has exhibited broad infectivity across numerous *Klebsiella* capsular types. A comprehensive analysis of the ΦK64-1 genome has unveiled the presence of eleven genes (S1–1 to S2–8), encoding proteins that share similarities with tailspike proteins or lyases. Another example is the jumbo phage jumbo bacteriophage vB_KleM-RaK2 [57], which also displays infectivity toward *Klebsiella* bacteria. Sequencing and annotating the genome of jumbo bacteriophage vB_KleM-RaK2 revealed the presence of ten tail fiber proteins, predominantly associated with the morphologically different podoviruses. Another jumbo phage, Atu_ph07 [58], exhibits the presence of tail fibers and peculiar 'hairy' whiskers. Interestingly, most of the tail fibers of these phages have not been structurally analyzed in detail. Phage ϕKp24 [55] has an exceptional amount of RBPs, allowing it to infect a large number of capsule types. A combination of cryo-electron tomography, single-particle analysis, artificial intelligence, and machine learning revealed that the tail fibers of ϕKp24 are structurally highly heterogeneous [41]. It is interesting to note that phages with HBTSs are often jumbo phages with relatively large genomes (> 340.000 bp) and are therefore frequently overlooked during routine phage isolation protocols that select for smaller particles. In addition, over 250 jumbo phages have been isolated from Gram-negative hosts and only 11 jumbo phages have been found to infect Gram-positive bacteria [59]. Although not confirmed, these large genomes might explain the capacity and space for multiple tail fiber or tailspike proteins. Given that these large phages are energetically costly to make, it is understandable why they have evolved complex HBTS structures that broaden the host range for capsulated bacteria with highly variable surfaces.

Conclusion

Understanding RBPs from different phages in more detail provides valuable insights into the initiation and progression of phage infection. In the past, especially the flexible fibers have potentially escaped detection in some previously described phages since they are not resolved by traditional imaging approaches. This implies that these fibers may be more abundant than currently appreciated. Since their important role in the phage's infection cycle, future studies will need to consider these components and use appropriate methods for detection and analysis. With the advent of new structural methods and image analysis techniques, combined with advanced computational approaches such as Alphafold predictions, machine learning, and artificial intelligence, we will rapidly gain structural insights into the less well-understood head or tail fibers and tailspikes. This may also allow the detection of even more complex RBP architectures than the four-level HBTSs that have been described. For instance, neural networks can be trained to track the complex RBPs of HBTSs from cryo-EM tomograms, which would otherwise require extensive time-consuming quantitative analyses more prone to error. In addition, machine learning can potentially be used to identify depolymerases or predict phage-host interactions with improved precision [60,61]. Resolving finer details of these RBPs may facilitate the reprogramming of phage-host range by engineering novel or chimeric tail fibers or tailspikes that can recognize and bind to specific host receptors. Determining the structure and function of phage fibers and spikes is a promising and challenging research direction that will bring future benefits for medical and industrial applications.

Data Availability

No data were used for the research described in the article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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- •• of outstanding interest

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