Structure

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Multiple masks of a *Shigella* podophage

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In this issue of *Structure*, Subramanian et al. present the cryo-EM structure of *Shigella* podophage HRP29, which possesses a T7-like tail complex surrounded by six P22/Sf6-like tailspikes and two unique decoration proteins. These colorful masks of HRP29 record the frequent events of horizontal gene transfer during evolution.

Phages are the most abundant and diverse biological entities on our planet with an estimated number of $\sim 10^{31}$.[1] Metagenomic analyses have identified many new phages in various environments, which greatly enhanced our understanding of their diversity.[2] Most phages isolated to date have a tailed morphology with a dsDNA genome. They are classified into three major families: *Myoviridae*, *Siphoviridae*, and *Podoviridae*. The key components in most tailed phages have strong similarities such as sharing an HK97-fold for the major capsid proteins (MCPs) and a dodecameric portal ring that links the tail to the capsid. In contrast, the tail that is pivotal for host recognition and genome delivery usually exhibits great diversity.[2] In the case of podophages, T7 features a tail adaptor and a nozzle that link the tail fibers, whereas P22 and Sf6 contain a tail needle and a needle head that anchor the tailspikes.[3,4]

In their elegant paper, Kristin Parent and colleagues report the cryo-electron microscopy (cryo-EM) structure of *Shigella* podophage HRP29,[5] and reveal a couple of distinct features for the assembly of an intact HRP29 virion (Figure 1). HRP29 has an unconventional hybrid tail, which is a fusion of the T7-like tail complex and Sf6-like tailspikes and a novel tailspike adaptor protein. Moreover, the mature capsid of HRP29, but not the procapsid, contains two novel decoration proteins, gp47 and gp48 on the outer surface, which stick to the inter-capsomer junctions and thereby reinforce the capsid stability. In contrast, the HRP29 procapsid harbors a scaffolding protein that is located at the inner surface of each capsomer, initiating the capsid assembly. These findings provide insights into the intricate architecture of HRP29 and highlight the structural versatility and adaptability of phages required for host recognition and infection.

In contrast to the previously reported structures of podophages, such as T7,[6] Sf6,[4] P22[3] and Pam1,[7] HRP29 distinguishes itself by a unique tail architecture comprising the portal gp35, adaptor gp39, nozzle gp40, tailspike adaptor gp44, and tailspike gp52. The portal, adaptor, and nozzle proteins are structurally similar to those of phage T7 with a similar modular organization. However, unlike T7 that is characterized by six tail fibers linked to the adaptor and nozzle proteins, HRP29 lacks the tail fibers despite sharing similar adaptor and nozzle proteins. Instead, HRP29 contains a unique trimeric tailspike adaptor gp44 that connects six tailspikes to the adaptor and nozzle. However, this tailspike adaptor has not been observed in conventional podophages such as Sf6, P22, and Pam1, whose tailspikes are directly attached to the adaptor and needle head via a distinct domain of the tailspike. On the other hand, the presence of a T7-like tail complex and functional tailspikes instead of tail fibers has been previously observed in phages K1-5, K1E, and SP6, which contain two sets of tailspikes. A common theme utilized by these phages is the presence of a tailspike adaptor protein that has evolved to contain an N-terminal domain similar to the T7 tail fiber that can dock to the tail complex and a unique C-terminal domain facilitating interactions with the tailspikes.

Both HRP29 and Sf6 are able to infect the *S. flexneri* strain of serotype Y, indicating that they most likely recognize the host via similar modules that constitute the tailspikes. Notably, structure predictions using AlphaFold2 reveal an overall structural similarity between the HRP29 and Sf6 tailspikes. Furthermore, both tailspikes share highly conserved active-site glutamate and aspartate residues, suggesting a similar putative catalytic mechanism for degrading the host receptor to facilitate host recognition. The major difference between these two tailspikes lies in the N-terminal region. The HRP29 tailspike is much shorter (508 residues), compared to the Sf6 tailspike (623 residues), which contains an N-terminal extension predicted to fold into a head-binding domain for the recognition of the needle head. Additionally, the N-terminal 41 residues of the HRP29 tailspike, which are involved in the interactions with the tailspike adaptor, diverge from their counterparts in the Sf6 tailspike that directly interact with the needle head.

Cement/decoration proteins are widely present in various phages and reinforce the stability of the mature capsid. These proteins exhibit various oligomerization states and diversity in their structures and the motifs they contain such as the β-tulip, β-sandwich, β-tadpole, Ig-like fold, and the knotted α-helical bundle.[8] The intact structure of HRP29 has revealed two small decoration proteins, gp47 and gp48, that are localized on the outer surface of the mature capsid. Notably, these proteins are absent from the procapsid, suggesting their involvement in post-maturation assembly and/or thermal stability. In contrast, some phages, such as HK97, lack decoration proteins. The capsomers of HK97 are stabilized by disulfide bonds formed by an autocatalytic covalent crosslinking reaction.[9] Structural analysis showed that gp47 adopts a helical bundle structure of four α-helices, whereas gp48 forms a dimer with each subunit comprising two stacked α-helices. The gp48 dimer shares a similar structure with the gp47 monomer, with the two helices

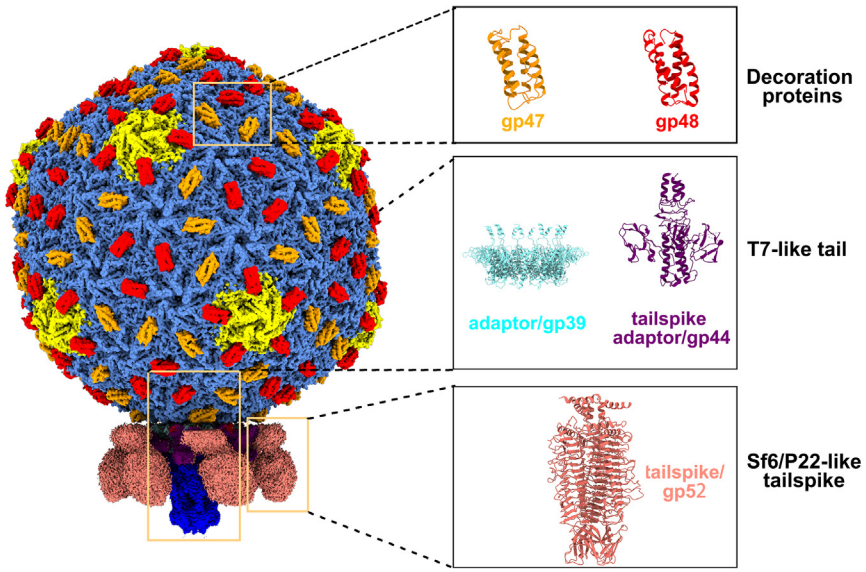

Figure 1. Cryo-EM map showing the overall architecture of an intact HRP29 virion
The zoomed-in views of the unique structural components are shown on the right. Structures of the decoration proteins, adaptor, tailspike adaptor, and tailspike are shown as cartoon representations, and the color scheme matches that of the cryo-EM map. The structure of the trimeric tailspike gp52 was predicted by AlphaFold2.

interacting with the capsid at a similar site. Notably, the cement/decoration proteins might have additional functions besides their role in stabilizing the capsid. For example, the cement protein gp6 of podophage Pam1, that exhibits structural similarities with the β-sandwich motif in its tailspike, potentially contributes to interactions between phages and the host cell's carbohydrates.[7] It also indicates that the presence of cement/decoration proteins might be driven by horizontal gene transfer from the phage's own or the host genome. Further studies are needed to explore the broader functional repertoire of these proteins in phages, which will provide more insights into the evolutionary advantages that are gained through the incorporation of diverse cement/decoration proteins.

In summary, the cryo-EM structure of the intact HRP29 virion reveals a different modular assembly pattern for phages, and it expands our understanding of phage genetic exchange in response to selective pressure. Compared to Sf6, which has a separate tailspike and tail needle, the hybrid tail in HRP29 combines the tailspike with a nozzle, which allows HRP29 to employ diverse modules for effective infection. Further studies are needed to elucidate the infection mechanism of HRP29, which will help us to better understand the prevalence and significance of such hybrid structures in nature. In addition, further comparative genomics and metagenomic studies might lead to the discovery of other phages with a hybrid architecture. In particular, exploring the selective pressures that drive the genetic diversity of phages with the integration of various structural modules will provide further insights into the evolutionary landscape of HRP29-like phages. A better understanding of the interplay between phages and their hosts will guide the synthesis of artificial phages with enhanced functionalities and for biotechnological applications.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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