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Phages must overcome the bacterial cell envelope to inject their genome into host cells and establish infection. Most phages use a tail to achieve this. The phage tail is a complex, multiprotein structure that mediates attachment, digestion and penetration of the cell wall and genome ejection. Little is known about how the tail breaches the last barrier that protects the cytoplasm of the host cell, the bacterial cellular membrane. Xu *et al.* now propose, based on crystal structures, cryo-electron microscopy (cryo-EM) images and protein sequence alignments, that phages use the tip of the tail to form a pore through which the genome enters bacterial cells.

In bacteriophage ϕ29, gene product 9 (gp9) sits at the distal tip of the tail and, together with gp13, it forms the tail knob, which has been implicated in membrane penetration. This placement makes it likely that gp9 is crucial for breaching the membrane barrier. Therefore, Xu *et al.* determined the crystal structure of gp9 and of a mutant gp9, in which a disorderd region, 74 amino acids long, had been deleted. Both the full-length and the mutant gp9 proteins formed hexameric, cylindrical tubes. The hexamer built by the mutant gp9 had a free inner channel with a diameter of 40 Å, which is wide enough to allow double-strand DNA to pass through. By contrast, in full-length gp9 hexamers, this channel was obstructed by the disorderd polypetide chains, which formed long loops (termed L loops by the authors) that started at the tip of the tube and bent back to fill two-thirds of the channel. Interestingly, the L loop contains many hydrophobic residues and several short helices, in a similar pattern to the HIV fusion peptide, which inserts into host cell membranes to mediate viral fusion.

When DNA ejection was induced by low pH, ϕ29 bacteriophages aggregated at their tail tips, a process that can be explained by exposure of the hydrophobic L loops during ejection and subsequent aggregation with L loops of close-by bacteriophages. Furthermore, cryo-EM images of post-ejection bacteriophages showed a distal cone-shaped structure at the tip of the tail, which was not present before genome ejection. The same cone-shaped structure was visible in cryo-EM images of bacteriophages that had ejected their DNA into liposomes. The cone-shaped structure was inserted into and spanned the whole width of the liposome membrane, and the liposomes contained dense DNA. A channel was not directly visible, probably because some DNA still remained in the cone-like structure. However, when DNA was completely removed through gradient purification, it became clear that the cone-shaped structure formed a pore. The pore-forming activity was confirmed in dye-release experiments, in which dye-filled liposomes were exposed to ϕ29 bacteriophages. Together, these experiments show that gp9 forms a tube at the end of the bacteriophage tail, which is blocked by a plug consisting of the hydrophobic L loops. During DNA ejection, these L loops exit the tail channel and insert into the bacterial cell membrane to form a pore. Genome sequence analyses revealed that several other bacteriophages, including the long-tailed bacteriophage T4, and even eukaryotic viruses, such as HIV and influenza virus, contain sequences that resemble the L loop sequence.

The discovery that the tail protein gp9 of bacteriophage ϕ29 contains a hydrophobic, pore-forming peptide suggests that prokaryotic and eukaryotic viruses use similar mechanisms to overcome the membrane barrier that protects host cells from viral infection.

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> “During DNA ejection, these L loops exit the tail channel and insert into the bacterial cell membrane to form a pore”

ORIGINAL ARTICLE Xu, J. *et al.* The bacteriophage Φ29 tail knob protein possesses a pore-forming loop for cell membrane penetration. *Nature* http://dx.doi.org/10.1038/nature18017 (2016)
FURTHER READING Molineux, I. J. & Panja, D. Popping the cork: mechanisms of phage genome ejection. *Nat. Rev. Microbiol.* **11**, 194–204 (2013)