VIRAL INFECTION

Penetration of non-enveloped viruses

Cell entry mechanisms of the non-enveloped viruses bluetongue and rhesus rotavirus are delineated using structural and molecular biology.

Yves Gaudin

To initiate an infection, a virus must deliver its genome into a host cell. Although entry is well understood for enveloped viruses[1,2], less is known about the entry machinery of eukaryotic non-enveloped viruses. Studies published in *Nature*[3] and *Nature Microbiology*[4] apply cryo-electron microscopy (cryo-EM) to pinpoint the molecular mechanisms underlying cell entry for two double-stranded (ds)RNA reoviruses: bluetongue virus (BTV), which is an endemic livestock pathogen in several countries[4], and rhesus rotavirus (RRV), which causes severe acute diarrhoea in young children[3].

The cell membrane poses a physical barrier to viral entry. Enveloped viruses, in which the viral genome and associated proteins are encased in a lipid bilayer, enter cells through the fusion of viral and cellular membranes[1]. The glycoproteins that comprise the fusion machinery of several enveloped viruses have been characterized in detail[1,2]. Fusion is catalysed by viral glycoproteins undergoing conformational changes to expose hydrophobic motifs that interact with the target membrane to initiate fusion. Non-enveloped viruses enter cells via an endocytic pathway and have to perforate the endosomal membrane. It has been difficult to determine the molecular details of those processes because the penetration machinery is only functional in the context of the whole virus particle. Characterization of non-enveloped viruse entry has been made possible by recent advances in cryo-EM.

Reoviruses package segmented genomes into icosahedral capsids that have three

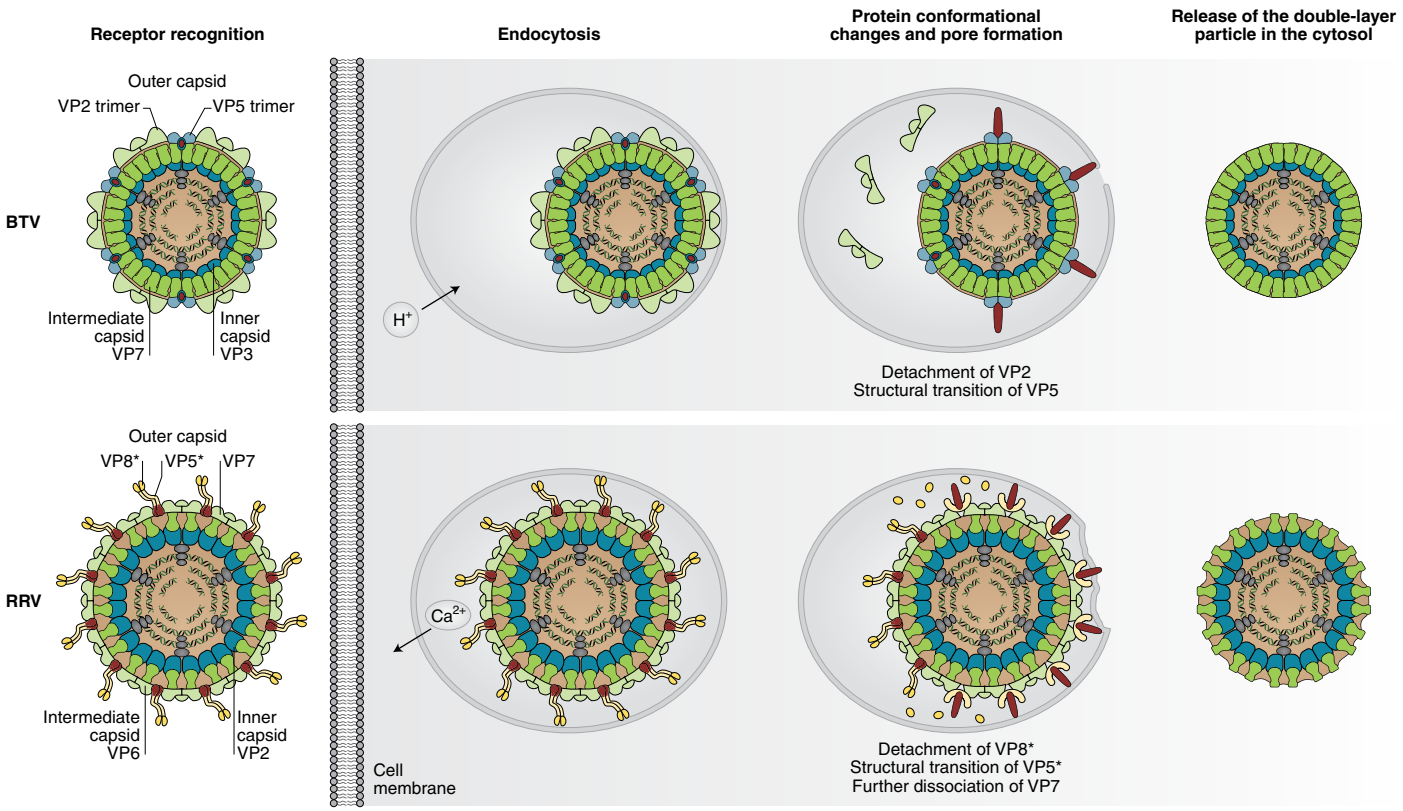

Fig. 1 | Mechanisms of BTV and RRV entry into host cells. After receptor attachment, the viral particle enters the cell via the endocytic pathway. Acidification of the endosomal compartment triggers BTV VP2 detachment and activates VP5. For RRV, the decrease in Ca^{2+} concentration in the endosome destabilizes the VP7 shell, induces the release of VP8* and triggers the VP5* conformational change. These changes induce the perforation of the membrane and release of the DLP in the cytosol. For both RRV VP5* and BTV VP5, there is a partial reversal of the protein's conformation, and the domains in red, which are buried in the native conformation, are projected outside the viral particle.

protein layers[5,6] (Fig. 1). The function of the outer layer is to deliver the viral core (also known as the double-capsid particle or double-layer particle (DLP)) that it surrounds into the cell cytoplasm. The core contains all of the machinery required to transcribe the genome segments and cap the viral mRNAs and extrude them into the cytosol. For both BTV and RRV, the outer layer contains two proteins. In rotaviruses[5], including RRV, those proteins are VP7, a Ca^{2+}-stabilized trimer forming the outer shell, and VP4, an atypical asymmetric trimer constituting the spikes that protrude from the virion surface. For the virion to become fully infectious, rotavirus VP4 must be cleaved into two fragments (VP8* and VP5*) by trypsin-like proteases after virion release in the intestinal lumen. VP8* mediates viral attachment to a cellular receptor, whereas VP5* perforates the lipid bilayer of the endosome. For BTV[6], the proteins that comprise the outer layer are VP2 (the attachment protein) and VP5 (the penetration protein). Both BTV and RRV exploit particular features of the endosomal pathway[7] that trigger conformational changes in their outer shell, enabling penetration. BTV takes advantage of the acidification of the endocytic vesicle, whereas rotaviruses take advantage of the concomitant decrease in calcium concentration, which destabilizes the VP7 shell.

Using cryo-EM, Xia et al.[4] reveal the details of the conformational change(s) at the surface of purified BTV briefly incubated with liposomes at pH 5.5. They used a symmetry-guided sub-particle reconstruction and classification workflow to sort out and solve four near-atomic-resolution structures of the virion at distinct stages of its structural transition. They show that VP2 detaches from the viral particle and VP5 undergoes a major conformational change, triggered by the protonation of clustering histidines forming a pH-sensitive molecular switch[8]. VP5 structural rearrangements result in the formation of a long stalk made of a coiled-coil bundle of six helices. At the tip of the stalk is a motif, composed of basic and hydrophobic residues, that may bind negatively charged lipids.

To obtain a three-dimensional representation of individual interaction events between BTV and a target membrane, the authors switched to cryo-electron tomography (cryo-ET). They applied purified BTV virions to grids, added a liposome-containing solution at pH 7.5, and washed the mixture first at pH 5.5 and then at neutral pH to mimic the successive stages of viral release in the cell cytosol. They found that the length of the six-helix stalk decreased from 19.5 to 15.5 nm after interacting with (or bumping into) a target membrane, suggesting that the tip of the stalk is inserted into the liposome bilayer. They also obtained images consistent with the creation of a single pore, which progressively expanded outward from the interaction site between the stalk and the liposome. This led them to propose a model for the release of the BTV core into the cytoplasm (Fig. 1), in which endosome acidification triggers VP2 detachment and the structural transition of VP5, which then interacts with the endosomal membrane, leading to pore formation.

Using cryo-EM and cryo-ET, Herrmann et al.[3] analysed the structural rearrangements of RRV spikes on the surface of purified virions (Fig. 1) following incubation at pH 11, which artificially triggers a conformational change that mimics the change that occurs in the endosome[9]. After exposure to alkaline conditions, a second conformation of the RRV spike, which the authors called the 'reversed conformation', was observed, as well as the initial asymmetric trimer made of VP5* and VP8*. In this reversed conformation, VP8* is no longer visible and VP5* adopts a three-fold symmetric structure in which three β-barrel domains, with hydrophobic loops exposed, surround a central trimeric coiled coil. The organization of VP5* in the reversed conformation is similar to that of a previously reported crystal structure of a fragment of purified recombinant VP5 (ref. [10]). The conformation is said to be 'reversed' because the $helix_{491–525}$ that forms the coiled coil and the C-terminal 'foot domain' of VP5, which is initially buried between the VP6 and VP7 proteins, are projected outside of the viral particle, owing to a 180° reorientation of the $helix_{491–525}$.

The authors then characterized the refolding pathway of VP5*. They used a recoating protocol to form triple-layer virions by incubating DLPs with recombinant VP4 and VP7. They recoated DLPs with a double cysteine mutant (S567C/A590C) of VP4, forming a disulfide bridge that locks the VP5* foot domain in its native folded structure. This mutant adopts an intermediate conformation containing three outward-projecting β-barrel domains but no central coiled coil. Taking these data together, Hermann et al.[3] propose that dissociation of VP8* from VP5* allows the transition to an intermediate state, resembling a foot-locked structure, which initiates the interaction with the target membrane. Then, the unfurling of the foot, the formation of the coiled-coil and the subsequent interaction of the foot with the target membrane enables penetration. This model is consistent with lower-resolution cryo-ET reconstructions of RRV entering host cells.

The structural analyses presented by Hermann et al. and Xia et al. together provide the most detailed molecular mechanisms for cell entry by non-enveloped dsRNA or dsDNA viruses to date. Refolding of the penetration proteins proceeds via the formation of an elongated trimeric coiled coil, which is reminiscent of the refolding of class I viral fusion glycoproteins[1]. Questions still remain about the refolding pathway of these proteins and the molecular bases of their interaction with the membrane and how pores are formed.

Cryo-ET, combined with integrative approaches, is reaching the stage where it can provide near-atomic resolution to in situ imaging, which will make it possible to definitively validate the function of these machineries in a cell. Understanding the molecular basis of the penetration mechanisms of non-enveloped viruses is a prerequisite for therapeutic target identification and may pave the way to designing entry-blocking therapies to treat viral diseases. ❐

Yves Gaudin ✉
Institute for Integrative Biology of the Cell (I2BC), CEA, CNRS, Université Paris-Saclay, Gif-sur-Yvette, France.
✉*e-mail: yves.gaudin@i2bc.paris-saclay.fr*

Published online: 26 October 2021
https://doi.org/10.1038/s41564-021-00991-z

References

1. Harrison, S. C. *Virology* **479–480**, 498–507 (2015).
2. Benton, D. J., Gamblin, S. J., Rosenthal, P. B. & Skehel, J. J. *Nature* **583**, 150–153 (2020).
3. Herrmann, T. et al. *Nature* **590**, 666–670 (2021).
4. Xia, X., Wu, W., Cui, Y., Roy, P. & Zhou, H. *Nat. Microbiol.* https://doi.org/10.1038/s41564-021-00988-8 (2021).
5. Rodríguez, J. M. & Luque, D. *Adv. Exp. Med. Biol.* **1215**, 45–68 (2019).
6. Roy, P. *Curr. Opin. Virol.* **24**, 115–123 (2017).
7. Huotari, J. & Helenius, A. *EMBO J.* **30**, 3481–3500 (2011).
8. Zhang, X. et al. *Nat. Struct. Mol. Biol.* **23**, 74–80 (2016).
9. Pesavento, J. B., Crawford, S. E., Roberts, E., Estes, M. K. & Prasad, B. V. V. *J. Virol.* **79**, 8572–8580 (2005).
10. Dormitzer, P. R., Nason, E. B., Prasad, B. V. V. & Harrison, S. C. *Nature* **430**, 1053–1058 (2004).

Competing interests

The author declares no competing interests.