# Penetration of non-enveloped viruses

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## Standfirst

Eukaryotic non-enveloped viruses enter the host cell through an endocytic pathway and perforate the endosomal membrane to release their genome in the cytoplasm. Two recent studies illuminate the molecular mechanisms underlying those processes for dsRNA enveloped viruses.

## Main text

To deliver their genome into the host cell, viruses must cross the physical barrier formed by the cell membrane. For enveloped viruses which are surrounded by a lipid bilayer, this step involves fusion between the viral and cellular membranes[1]. The glycoproteins making the fusion machinery of several enveloped viruses have been characterized in detail[1,2]. This showed that fusion is catalyzed by a huge conformational change of those glycoproteins. During the transition, hydrophobic motifs are exposed and interact with the target membrane to initiate the fusion reaction.

Less is known about the entry machinery of eukaryotic non-enveloped viruses. Those viruses enter the host cell through an endocytic pathway and perforate the endosomal membrane. Two recent articles published in *Nature* and *Nature Microbiology*[3,4] present cryo-electron microscopy studies that illuminate the field by revealing the molecular mechanisms underlying those processes for two dsRNA viruses belonging to the reoviridae family: bluetongue virus (BTV, which is an endemic livestock pathogen in several countries)[3] and rhesus rotavirus (RRV, a member of the rotavirus genus which gathers pathogens that are

responsible for severe acute diarrhea in young children worldwide)[4]. These viruses package segmented genomes into icosahedral capsids having three protein layers[5,6] (Figure 1). The function of the outer layer is to deliver the viral core (also called double capsid particle or double layer particle) that it surrounds into the cell cytoplasm. The core contains all the machinery required to transcribe the genome segments, cap the viral mRNAs and extrude them into the cytosol.

For both viruses, the outer layer is made of two proteins. For rotaviruses[5], those proteins are VP7, a $Ca^{2+}$-stabilized trimer forming the outer shell, and VP4, an atypical asymmetric trimer constituting the spikes that protrude at the virion surface. For the virion to become fully infectious, rotavirus VP4 must be cleaved to generate two fragments (VP8* and VP5*) by trypsin-like proteases after virion release in the intestinal lumen. VP8* is responsible for viral attachment to its cellular receptor, whereas VP5* is responsible for the perforation of the lipid bilayer of the endosome. For BTV[6], the proteins that constitute the outer layer are VP2 (the attachment protein) and VP5 (the penetration protein). Both viruses exploit distinct features of the endosomal pathway to achieve the penetration step. Rotaviruses take advantage of the decrease in calcium concentration, which destabilizes the VP7 shell, whereas BTV takes advantage of the pH decrease to perforate the endosomal membrane.

Using state of the art cryo-electron microscopy, Xia et al.[3] reveal the details of the conformational change at the surface of BTV upon low pH exposure (Figure 1). They provide four near-atomic resolution structures of the virion at different stages of the process. They show that VP2 detaches from the viral particle, and that VP5 undergoes a major conformational change that is triggered by the protonation of clustering histidines forming a pH-sensitive molecular switch[7]. Globally, VP5 structural rearrangements result in the formation of a long stalk made of a coiled-coil bundle of six helices at the tip of which is located a motif, composed of basic and hydrophobic residues, that potentially binds negatively charged lipids. Using, cryo

electron tomography (Cryo-ET), they further explored the interaction of BTV with liposomes. The length of the stalk decreases from 19.5 to 15.5 nm after interacting with a target membrane, suggesting that the tip of the stalk is inserted into the liposome bilayer. Finally, they observe the creation of a single pore, which progressively expands, at the interaction site between the stalk and the liposome. This leads them to propose a model for the release of BTV core into the cytoplasm.

Using similar techniques, Herrmann et al.[4] investigated the structural rearrangements of RRV spikes at the surface of virions (Figure 1). The conformational change was artificially triggered by increasing the pH to 11[8]. After such a treatment, besides the native-like asymmetric trimer made of VP5* and VP8*, a second conformation, that the authors called the reversed conformation, is also observed. In this conformation, VP8* is no more visible and VP5* adopts a three-fold symmetric structure in which three β-barrel domains, exposing hydrophobic loops, surround a central trimeric coiled-coil (an organization that is similar to a previous crystal structure of a fragment of VP5 in solution[9]). The conformation is said « reversed » because the $helix_{491-525}$ making the coiled coil and the C-terminal « foot domain » of VP5, initially buried between the VP6 and VP7 proteins, now project ouside the viral particle, thanks to a 180° reorientation of the $helix_{491-525}$. Moreover, using a clever recoating protocol of double layer particles with recombinant VP4 and VP7, they show that a double cystein mutant, locking VP5* foot domain in its native folded structure, adopts an intermediate conformation containing three outward projecting β-barrel domains but no central coiled coil. Thus, they propose that dissociation of VP8* from VP5* allows the transition to an intermediate state, resembling the 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 achieve the penetration process. This model is in agreement with cryo-ET reconstructions of RRV entering their host cells[4].

Those two studies give the most detailed molecular mechanisms so far for penetration of dsRNA or dsDNA viruses. They reveal that the refolding of the penetration proteins proceeds through the formation of an elongated trimeric coiled coil, which is reminiscent of the refolding of class I viral fusion glycoproteins[1]. There are still many questions regarding the refolding pathway of those proteins, the molecular bases of their interaction with the membrane and how all of this leads to a pore formation. The already impressive and future progress of Cryo-ET should make it possible to definitively validate the functioning of these machineries within the cell itself as well as to extend those analyzes to other non-enveloped viruses. Finally, the molecular description of the penetration mechanisms of non-enveloped viruses paves the way to design novel vehicles for intracellular delivery of molecules with biological activity.

**Competing interests**

The author declares no competing financial interests.

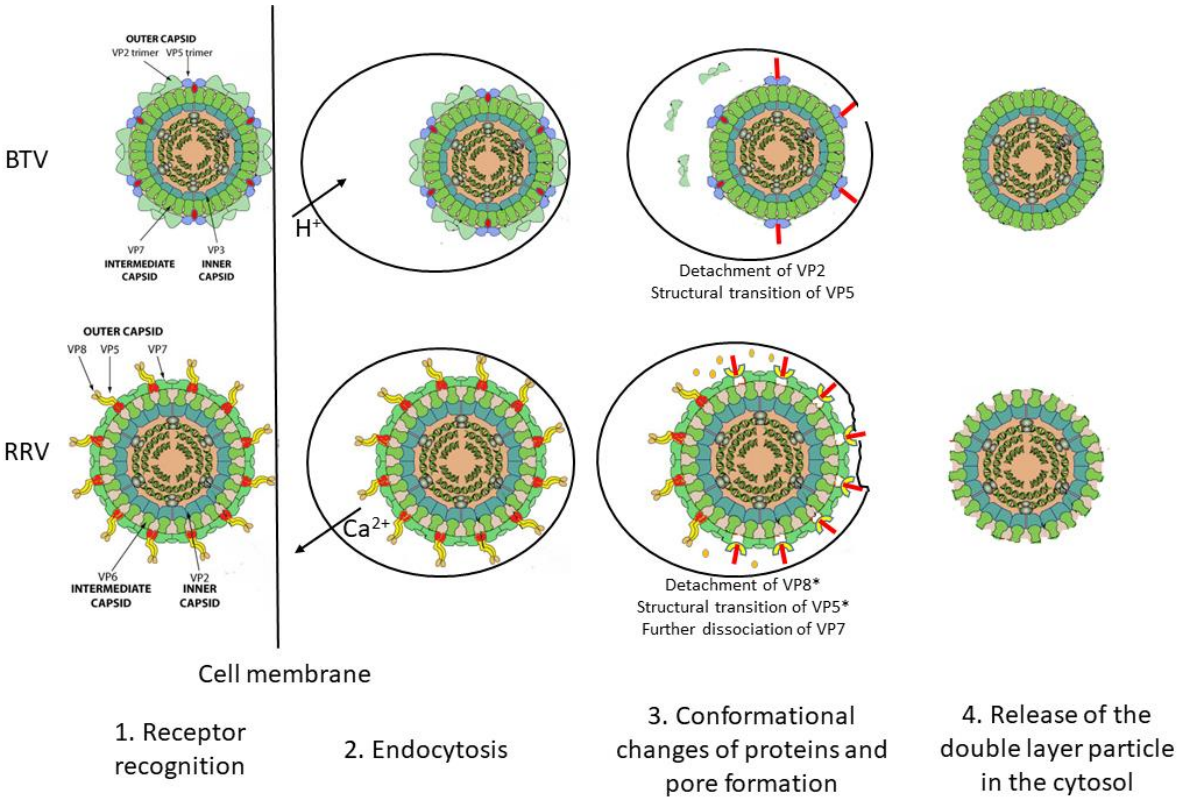


Fig. 1: **Mechanisms of BTV and RRV entry into the host cell.** After receptor attachment, the viral particle enters the cell via the endocytic pathway. Acidification of the endosomal compartment triggers BTV VP2 detachment and activates VP5. For RRV, the decrease of $Ca^{2+}$ contentration in the endosome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

## References


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