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# Bluetongue virus structure and assembly

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Bluetongue virus (BTV) is an insect-vectored emerging pathogen of wild ruminants and livestock in many parts of the world. The virion particle is a complex structure of consecutive layers of protein surrounding a genome of ten double-stranded (ds) RNA segments. BTV has been studied as a model system for large, non-enveloped dsRNA viruses. Several new techniques have been applied to define the virus-encoded enzymes required for RNA replication to provide an order for the assembly of the capsid shell and the protein sequestration required for it. Further, a reconstituted *in vitro* system has defined the individual steps of the assembly and packaging of the genomic RNA. These findings illuminate BTV assembly and indicate the pathways that related viruses might use to provide an informed starting point for intervention or prevention.

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## Introduction

Bluetongue virus (BTV) is a member of the Orbivirus genus within the *Reoviridae* family, one of the largest families of viruses. These viruses represent a diverse group of double-stranded (ds) RNA viruses with capsids consisting of 1–3 protein layers and infect a wide range of hosts including human, animal, plant and insect. Consequently, the host receptor-binding proteins of these viruses, which occupy the outer layer of the virus particle, are largely non-homologous. Orbiviruses represent an intriguing system for study. They are vectored to particular vertebrate species (*e.g.*, sheep, cattle, horses, deer, *etc.*) by arthropods (gnats, ticks, or mosquitoes depending on the virus), hence determining their geographic distribution. BTV, with 27 serotypes, is one of the most widespread animal pathogens and acts as an important representative of this class of large non-enveloped viruses. In recent years considerable progress has been made in determining the BTV structure at the atomic level, providing new insights into the structure–function relationships among the proteins of the virus particle and in virus assembly/disassembly. The introduction of mutations into the replicating viral genome by reverse genetics (RG) systems, first developed for BTV, has opened up a new opportunity to dissect the various stages of virus replication and assembly. Further, the development of novel assays has unravelled key aspects of the genome packaging mechanism. Consequently, BTV represents one of the best characterized viruses and selected aspects of its entry, replication and assembly are reviewed here.

## Overview of the replication cycle

Infection by the BTV particle (~550S) is established when the inner capsid of the double-capsid particle, the 'core', translocates across the endosomal membrane following virus uptake, a process performed by the two outer capsid proteins, VP2 and VP5 [1–6]. The larger VP2 protein (110 kDa) binds to surface glycoproteins of unknown identity and facilitates clathrin mediated endocytosis of the virion, while the VP5 protein (60 kDa) penetrates the host cell membrane and delivers the 470S core particle into the host cytosol. The process has two distinct stages: VP2 senses the pH in the early endosome (6.5–6.0) and detaches while the remaining particle continues to the late endosome, where VP5 senses a lower pH (~5.5) and gains membrane 'fusion' activity, allowing the core to escape through the endosomal membrane. The final product of disassembly for all members of the *Reoviridae* is the transcriptionally active double-capsid particle able to initiate transcription of the genomic RNAs. The surface layer of the BTV core is formed by VP7 which coats the internal VP3 layer which in turn encapsidates the viral tri-protein transcriptase complex (TC), VP1 (polymerase), VP4 (capping enzyme) and VP6 (helicase/RNA packaging) and the 10 genomic dsRNA segments (S1–S10) [7,8]. Within the core, each segment is repeatedly transcribed and modified by the TC and ten transcripts, each with a 5′ cap 1 structure, but lacking polyadenylation at their 3′ terminus, are released into the cytosol. These ssRNAs serve as mRNA for the synthesis of viral proteins within the cytoplasm and later also act as templates for dsRNA genome synthesis. In addition to the seven structural proteins, four non-structural proteins, NS1–NS4 are synthesized in infected cells, each supporting virus growth at different stages. Of these, NS1 preferentially promotes translation of BTV ssRNAs enhancing viral protein synthesis, while NS2 forms a fibrillar network, viral inclusion bodies (VIBs), which

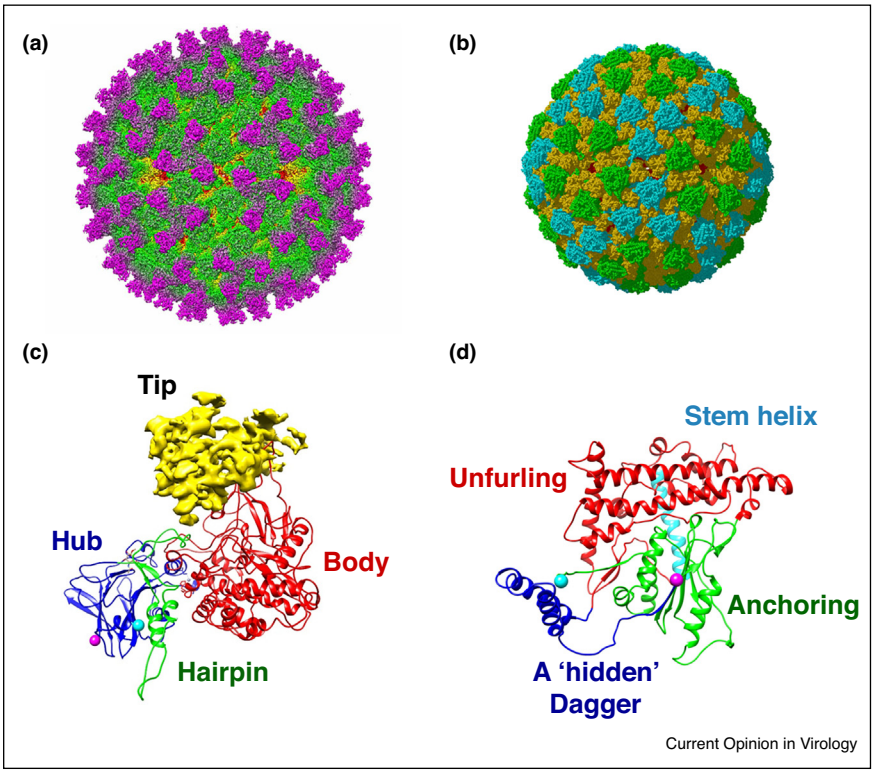
recruit viral ssRNAs and protein components required for genomic packaging, replication and core assembly [2,8–10]. Following assembly newly synthesised cores are released from VIBs and utilise the intracellular vesicular sorting and exocytosis pathway in doing so [11–16]. This process is facilitated by NS3, and the outer capsid proteins VP5 and VP2 are acquired in the process to produce mature particles that egress from the host cell and propagate the infection.

## BTV outer capsid

Cryo-electron microscopy (Cryo-EM) and image analysis of BTV have revealed the well-ordered structural arrangement of the virion. The outer shell has 60 triskelion-like VP2 spikes, surrounding 120 globular trimers of VP5, the membrane penetration protein [17–20]. The detailed structures of these two proteins and the whole particle have been recently defined at near-atomic resolution (3.5 A) suggesting how each may function during virus entry into the cell (Figure 1) [21•]. The receptor binding VP2 monomer is divided in four distinct domains, a hub, a hairpin, a pyramid-shaped body and a highly flexible external tip, where host neutralizing antibodies bind. The hub domain has a 10-stranded lectin-like β-barrel fold and drives the monomer–monomer interaction of the trimer as well as possesses a sialic acid binding pocket. At the interface of the hub and the body domains a well-coordinated tetrahedron of a typical zinc-finger motif, CCCH, is found and is shown to be responsible for pH triggered VP2 conformational changes in the early endosome.

A series of biochemical and molecular studies have established that VP5 is the membrane penetration ('fusion') protein and the structural data corroborate this and reveal how VP5 may function [4,5,21•]. The VP5 trimers possess a highly compact globular fold predominated by α-helices with a central coiled-coil motif facilitating trimerization, analogous to membrane viral fusion proteins such as the stalk of Human Immunodeficiency Virus gp41. Each VP5 monomer has three distinct domains: a flexible dagger, which is sequestered in the canyons underlying the core surface, a helix-rich unfurling domain, and an anchoring domain with membrane-interaction elements. Notably, a β-meander motif in the anchoring domain contains a

**Figure 1**




CryoEM density map of the BTV virion obtained at 3.5-Å-resolution, shown as radially colored surface representation. The outer layer contains 60 VP2 trimers (magenta) and 120 VP5 trimers (green); a middle layer with 260 VP7 trimers (yellow); and an inner layer formed by 120 VP3 monomers (red) almost completely occluded by the VP7 layer. **(b)** The VP5 layer showing the two different trimer conformers in green and cyan. **(c & d)**. Ribbon model of the VP2 (c) and (d) monomer respectively with different domains colour-coded. The stem helix (cyan ribbon) of the unfurling domain is shown with the N and C termini indicated as magenta and cyan balls, respectively.