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### Authors

He, Yao  
Shivakoti, Sakar  
Ding, Ke  
et al.

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# In situ structures of RNA-dependent RNA polymerase inside bluetongue virus before and after uncoating

Yao He<sup>a,b</sup>, Sakar Shivakoti<sup>a,b</sup>, Ke Ding<sup>b</sup>, Yanxiang Cui<sup>a,b</sup>, Polly Roy<sup>c</sup>, and Z. Hong Zhou<sup>a,b,1</sup>

<sup>a</sup>Department of Microbiology, Immunology & Molecular Genetics, University of California, Los Angeles, CA 90095; <sup>b</sup>California NanoSystems Institute, University of California, Los Angeles, CA 90095; and <sup>c</sup>Department of Pathogen Molecular Biology, London School of Hygiene and Tropical Medicine, WC1E 7HT London, United Kingdom

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**Bluetongue virus (BTV), a major threat to livestock, is a multilayered, nonturreted member of the *Reoviridae*, a family of segmented dsRNA viruses characterized by endogenous RNA transcription through an RNA-dependent RNA polymerase (RdRp). To date, the structure of BTV RdRp has been unknown, limiting our mechanistic understanding of BTV transcription and hindering rational drug design effort targeting this essential enzyme. Here, we report the in situ structures of BTV RdRp VP1 in both the triple-layered virion and double-layered core, as determined by cryo-electron microscopy (cryoEM) and subparticle reconstruction. BTV RdRp has 2 unique motifs not found in other viral RdRps: a fingernail, attached to the conserved fingers subdomain, and a bundle of 3 helices: 1 from the palm subdomain and 2 from the N-terminal domain. BTV RdRp VP1 is anchored to the inner surface of the capsid shell via 5 asymmetrically arranged N termini of the inner capsid shell protein VP3A around the 5-fold axis. The structural changes of RdRp VP1 and associated capsid shell proteins between BTV virions and cores suggest that the detachment of the outer capsid proteins VP2 and VP5 during viral entry induces both global movements of the inner capsid shell and local conformational changes of the N-terminal latch helix (residues 34 to 51) of 1 inner capsid shell protein VP3A, priming RdRp VP1 within the capsid for transcription. Understanding this mechanism in BTV also provides general insights into RdRp activation and regulation during viral entry of other multilayered, nonturreted dsRNA viruses.**

dsRNA virus | reovirus | RNA-dependent RNA polymerase | transcription | cryoEM

**B**luetongue virus (BTV) is a member of the *Orbivirus* genus within the *Reoviridae* family, a large family of viruses that infect insects, plants, and animals, including humans (1). These nonenveloped viruses are characterized by a genome that comprises 9 to 12 segments of linear, double-stranded RNA (dsRNA) enclosed alongside multiple copies of an RNA-dependent RNA polymerase (RdRp) in an icosahedral capsid shell (2–4). They can be divided into 2 subfamilies, turreted (*Spinareovirinae*) and nonturreted (*Sedoreovirinae*), based on the presence or absence of turrets on their (inner) capsids.

As a nonturreted member of the family, BTV has 4 structural proteins organized into a triple-layered capsid: an outer layer composed of 60 triskelion-like VP2 trimers and 120 globular trimers of VP5 interdigitating one another, a middle layer of 260 VP7 trimers, and an inner layer formed by 60 dimers of the capsid shell protein VP3 (5–9). Ten genomic dsRNA segments are compacted inside the capsid shell alongside enzymatic proteins, including putative helicase VP6 (10, 11), mRNA capping enzyme VP4 (12, 13), and RdRp VP1 (6, 14, 15). During cell entry, the VP2 protein binds receptors on the host cell surface and facilitates clathrin-mediated endocytosis of the BTV virion, while the VP5 protein penetrates the cell membrane and releases the double-layered particle, the core, into the host cytosol (8, 16). The BTV core remains intact in the cytosol with 10 genomic dsRNA segments encapsidated while avoiding the antiviral defense

mechanisms of the host cell (14, 17). In the BTV core, the RdRp VP1 protein initiates endogenous transcription, using the negative strand of each genomic dsRNA segment as template, and then the newly synthesized positive-strand RNA transcripts are capped by capping enzyme VP4 and extruded into the host cytosol (14, 15, 18). These positive strands of RNA serve as mRNA for the translation of viral proteins within the cytoplasm. Subsequently, during virus assembly, the positive strands are packaged with newly synthesized capsid proteins to serve as the positive strand from which the complementary strand of the genomic dsRNA is replicated (19–21).

Icosahedral structures of the BTV virion and core have been determined to near-atomic resolution with X-ray crystallography and cryo-electron microscopy (cryoEM) (5, 8). However, what the structure of BTV RdRp VP1 is and how it interacts with the genome and other proteins are both unknown, significantly limiting our understanding of the RNA transcription mechanism inside BTV. The structures of RdRp and associated NTPase proteins have recently been determined by cryoEM for 2 turreted reoviruses: cytoplasmic polyhedrosis virus (CPV) (22, 23) and aquareovirus (ARV) (24, 25). However, as a nonturreted member

## Significance

**Bluetongue virus (BTV) is a double-stranded RNA (dsRNA) virus that often causes significant economic damage to the livestock industry. The RNA-based genome transcription of BTV during infection depends on its RNA-dependent RNA polymerase (RdRp) inside the capsid. Here, we report a structure of BTV RdRp derived from cryo-electron microscopy (cryoEM) and reconstruction methods. The structure reveals how BTV RdRp interacts with associated genomic dsRNA and capsid proteins. Structural comparison between RdRp in the transcriptionally incompetent BTV virion and transcriptionally competent BTV cores provides insights into the highly regulated mechanism of mRNA transcription initiation. This structure of BTV RdRp and our mechanistic findings could now be exploited to design antivirals against the bluetongue disease in the livestock farming industry.**

Author contributions: Z.H.Z. designed research; Y.H., S.S., K.D., Y.C., and P.R. performed research; Y.H. and Z.H.Z. analyzed data; and Y.H. and Z.H.Z. wrote the paper.

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Data deposition: The cryoEM density maps have been deposited in the Electron Microscopy Data Bank under accession codes [EMD-20398](#) (RdRp VP1 in BTV virion) and [EMD-20407](#) (RdRp VP1 in BTV core). The atomic coordinates have been deposited in the Protein Data Bank under accession codes [6PNS](#) (RdRp VP1 in BTV virion) and [6PO2](#) (RdRp VP1 in BTV core).

<sup>1</sup>To whom correspondence may be addressed. Email: [Hong.Zhou@UCLA.edu](mailto:Hong.Zhou@UCLA.edu).

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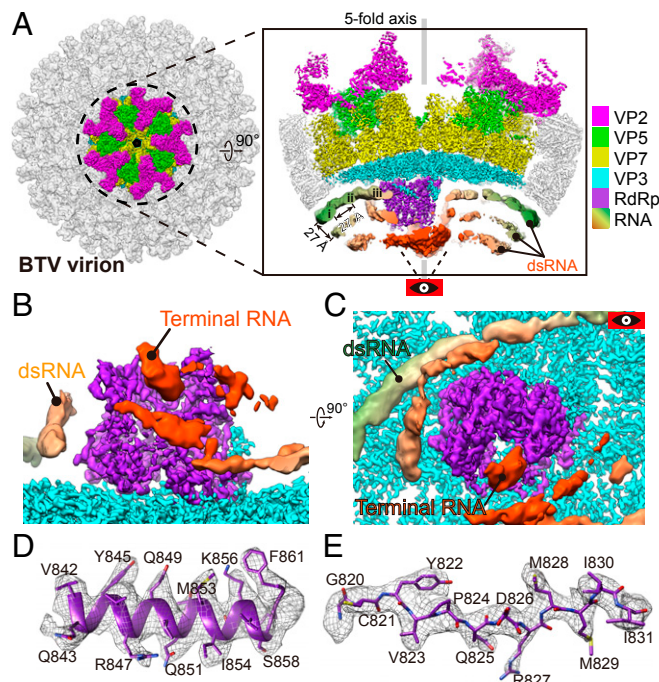
of the *Reoviridae*, BTV does not contain an NTPase homolog, suggesting a different mechanism of virial transcription and assembly. The only known RdRp structure from a nonturreted member of the *Reoviridae* is that of rotavirus (26). Although its location inside the capsid shell has been studied by cryoEM (27–29), there is only limited understanding of how capsid proteins and RNA interact with RdRp to activate and regulate transcription in situ.

In this study, we determined the in situ structures of BTV RdRp VP1 inside both the triple-layered BTV virion and the double-layered BTV core by cryoEM and subparticle reconstruction methods. Our asymmetric reconstructions of the BTV vertex region reveal that RdRp VP1 is anchored to the inside surface of the inner capsid shell via 5 asymmetrically arranged N termini of the 5 VP3 molecules around it. The structural changes of RdRp VP1 and its associated capsid proteins between the virions and the cores suggest that detachment of the outer proteins during viral entry induces both global movements of the capsid shell and local conformational changes of an interacting VP3, priming RdRp VP1 within the capsid for transcription. This mechanism provides general insights into RdRp activation and regulation during cell entry of multilayered, nonturreted reoviruses.

## Results

**Overall Structures.** To obtain the in situ structure of BTV RdRp VP1, we recorded cryoEM images (*SI Appendix, Fig. S1A*) of purified intact BTV virions with a direct electron detector and carried out single-particle analysis for 3D reconstruction (*SI Appendix, Fig. S2*). Icosahedral reconstruction reveals a flower-shaped, 5-fold symmetric density under each 5-fold vertex of BTV virion (*SI Appendix, Fig. S3A and B*), similar to those contributed by RdRp-containing transcriptional enzyme complexes in other members of the *Reoviridae* (25, 28). However, structural details of this RdRp density remained obscure due to symmetrization during icosahedral reconstruction, except for a few  $\alpha$ -helices (*SI Appendix, Fig. S3A and B*). To resolve the structure of RdRp VP1 and dsRNA genome around it, we boxed out each icosahedral vertex as a subparticle from particle images and carried out asymmetric subparticle reconstruction (*SI Appendix, Fig. S2*). Essentially, each subparticle's 5 possible symmetry-related orientations, predicted from the icosahedral symmetry, were classified to identify a single asymmetric orientation and then locally refined (30, 31). An asymmetric reconstruction (Fig. 1A) was obtained at an effective resolution of 3.7 Å by combining 244,063 vertex subparticles (*SI Appendix, Fig. S1C and D*).

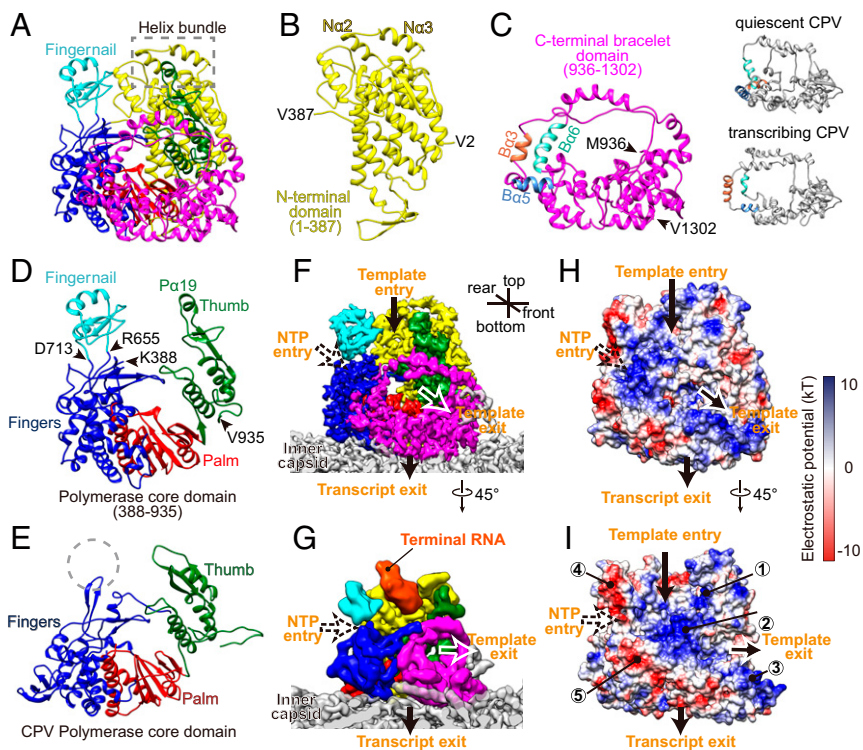
Our asymmetric subparticle reconstruction of the vertex region reveals the organization of BTV RdRp VP1 and dsRNA densities around it (Fig. 1A and *Movie S1*). One RdRp VP1 alone is anchored to the inner capsid shell by N termini of the 5 VP3 molecules surrounding the icosahedral 5-fold axis (Fig. 1A). This organization differs from that of the turreted members of the *Reoviridae*, whose capsid-anchored transcriptional enzyme complex structures are heterodimers of an RdRp and an NTPase (22–25). Above these internal structures are 3 layers of capsid proteins, including the inner capsid shell formed by VP3 dimers, the middle layer of VP7 trimers, and the outermost layer containing the receptor-binding protein VP2 and penetration protein VP5 (Fig. 1A and *Movie S1*). The structures of these capsid proteins resolved in our asymmetric reconstruction are similar to those previously resolved in icosahedral reconstructions (7, 8). Inside the capsid shell, the dsRNA genome exhibits as parallel fragments, which are tightly packed into several layers surrounding RdRp VP1 (Fig. 1A). Each dsRNA fragment is separated from its neighbors, in the same layer or in adjacent layers, by an average of ~27 Å (Fig. 1A). However, in general, the RNA densities are not well-resolved, especially the RNA fragments in front of RdRp VP1, as shown in Fig. 1B, indicating a mixture of different RNA conformations across different particles. One piece of RNA



**Fig. 1.** CryoEM reconstruction of the vertex region of BTV virion. (A) Superposition of the subparticle reconstruction of BTV vertex (colored) and the icosahedral reconstruction of BTV virion (gray), showing the relative locations of the genomic RNA and individual proteins. The RNA densities are lowpass-filtered and radially colored. See also *Movie S1*. (B and C) CryoEM density map of RdRp VP1 and associated RNA shown in 2 orthogonal views. Nonassociated RNA densities are removed for clarity. (D and E) Close-up views of an  $\alpha$  helix (D) and a loop (E) of RdRp VP1. Atomic models are shown as ribbons or sticks and cryoEM density as meshes.

density, labeled as terminal RNA, binds at the upper region of RdRp VP1 (Fig. 1B).

**Atomic Model of RdRp VP1.** Typical of a 3.7-Å-resolution cryoEM map, our subparticle asymmetric reconstruction resolves bulky amino acid side chains (Fig. 1D and E), allowing us to build an atomic model of BTV RdRp VP1 (Fig. 2, *SI Appendix, Fig. S1F*, and *Movie S2*). Each RdRp VP1 consists of an N-terminal domain (residues 1 to 387), a polymerase core domain (residues 388 to 935), and a C-terminal bracelet domain (residues 936 to 1302) (Fig. 2A–D and *SI Appendix, Fig. S4*). Although this domain organization is similar to that of the known RdRp structures of the *Reoviridae* (22, 24, 26, 32, 33), BTV RdRp VP1 contains a fingernail motif and a helix bundle motif, both of which have not been observed in any other viral RdRp structures (*SI Appendix, Fig. S5*). The polymerase core domain, sandwiched between the N-terminal domain and the C-terminal bracelet domains, consists of 3 subdomains, fingers, palm, and thumb, arranged in classical right-hand configuration (Fig. 2D). The catalytic site lies within a cavity in the center of RdRp VP1 and does not contain any visible RNA density (Fig. 2F), indicating that the polymerase remains quiescent in triple-layered BTV virion, as expected. Four channels connecting the catalytic cavity with the exterior are used for template entry (top), NTP entry (rear), transcript exit (bottom), and template exit (through the center of C-terminal bracelet domain in the front; Fig. 2F and G). Notably, the C-terminal bracelet domain of RdRp VP1 in the quiescent BTV virion adopts an open conformation with the template exit channel wide open, a conformation similar to that of the bracelet domain in quiescent and primed ARV (24) and transcribing CPV (Fig. 2C), both turreted reoviruses.



**Fig. 2.** In situ structure of RdRp VP1. (A–D) Ribbon representation of the atomic model of BTv RdRp VP1 showing in full (A) and as separate domains: N-terminal domain (B) C-terminal bracelet domain (C) and polymerase core domain (D). See also [Movie S2](#). (C, Right) Ribbon models of the CPV bracelet domain in both quiescent and transcribing states (22) are shown, with corresponding modules highlighted for comparison. (E) Ribbon model of CPV polymerase domain (22). Comparison between BTv (D) and CPV (E) polymerase domains shows the fingernail motif (cyan) atop the BTv fingers subdomain (blue). (F–G) CryoEM density maps of RdRp VP1 alone (F) and with terminal RNA binding (G) showing the 4 channels: template entry, template exit, NTP entry, and transcript exit. Densities in G are lowpass-filtered to better show the RNA density. (H–I) Coulomb potential surfaces of RdRp VP1 in 2 different views. Depictions of BTv RdRp VP1 in A, D, F, and H are all in the same orientation.

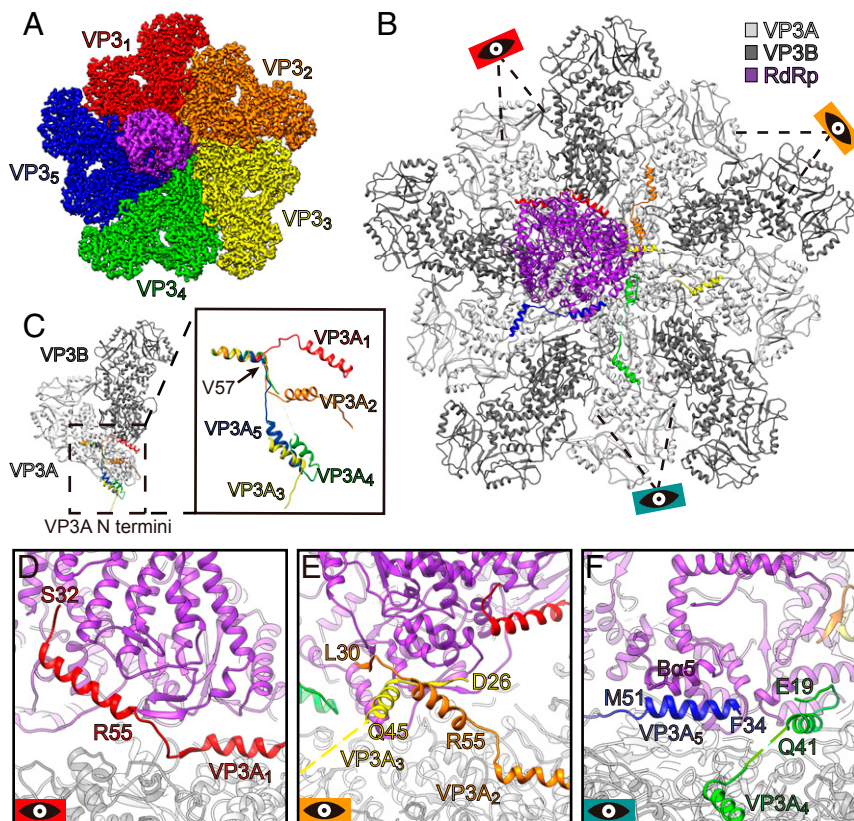
The fingernail motif (residues 655 to 713) sits atop the fingers subdomain of BTv RdRp VP1 (Fig. 2D), representing an insertion into the characteristic  $\beta$  hairpin in the fingers subdomain (Fig. 2E and [SI Appendix, Fig. S5](#)) of other viral RdRp structures known to date. Flanking 1 side of the template entry channel, the fingernail motif possesses opposite electrical charge properties on its 2 sides ([SI Appendix, Fig. S6](#)). The positively charged side ([SI Appendix, Fig. S6E](#)) attaches to the edge of the N-terminal domain ([SI Appendix, Fig. S6B](#)) via strong electrostatic interactions, whereas the negatively charged side faces the dsRNA genome around RdRp VP1 (Fig. 2I and [SI Appendix, Fig. S6D](#)). On the other side of the template entry channel is a helix bundle motif consisting of 3  $\alpha$ -helices from the N-terminal domain (Na2 and Na3 in Fig. 2B) and the palm subdomain (P $\alpha$ 19 in Fig. 2D), a motif also absent in the known RdRp structures of other members of the *Reoviridae* ([SI Appendix, Fig. S5](#)).

Our in situ structure of the RdRp with bound RNA suggests a likely path of RNA during transcription. At the entrance to the template entry channel, the terminal RNA has a skinny end that binds residue H40 ([SI Appendix, Fig. S7](#)) of the helix bundle motif (Fig. 2G). This skinny end of the terminal RNA is likely the 5' cap of the positive strand of a dsRNA genome segment. Such interaction between the 5' cap and histidine residue is also observed in the structure of ARV RdRp in the primed state (24). In the initial step of transcription, the 5' cap of the RNA positive strand may move further and interact with positively charged (region 1 in Fig. 2I) and aromatic (e.g., H183) residues in the cap-binding site ([SI Appendix, Fig. S7](#)), which is equivalent to the cap-binding sites observed in the RdRp structures of reovirus and rotavirus (22, 24, 26, 34). In such a scenario, the negative strand of the same terminal dsRNA could bind residue H48 on the other side ([SI Appendix, Fig. S7](#)) to facilitate splitting of the 2 RNA strands to initiate RNA transcription. Subsequently, the negative strand would slide through the template entry channel into the catalytic cavity to synthesize the nascent mRNA and exit through the template exit channel (Fig. 2F and G). On the surface of RdRp VP1, a contiguous positively charged strip (region 2 to 3

in Fig. 2I), lined by negatively charged strips (regions 4 and 5 in Fig. 2I), forms a track to guide the movement of the RNA positive strand, leading to the reannealing of 2 RNA strands outside the template exit channel. This RNA pathway is similar to those visualized in the in situ structures of rotavirus RdRp during transcription (34, 35).

**Interactions between the N Termini of 5 VP3A Molecules and RdRp VP1.** Our asymmetric subparticle reconstruction of the BTv vertex region resolves asymmetrically arranged N-terminal regions (residues are indicated in [SI Appendix, Fig. S8](#)) of the inner capsid protein VP3A (Fig. 3 and [Movie S2](#)), which, due to the imposition of symmetry, were invisible in both the icosahedral cryoEM reconstruction of the BTv virion (7, 8) and the X-ray crystal structure of the BTv core (5). The inner capsid of each BTv vertex consists of 5 VP3A-VP3B dimers (Fig. 3A). VP3A<sub>1–5</sub> are arranged into a star-shaped structure, with their conserved apical domains forming the mRNA exit pore along the 5-fold axis, whereas VP3B<sub>1–5</sub> are wedged between neighboring VP3As to complete the inner capsid shell (Fig. 3B). The newly resolved N-terminal fragment contains 1  $\alpha$ -helix connected to the rest of the VP3A model via a structurally variable loop (Fig. 3C). This variable loop allows the N-terminal segment of VP3A to adopt different conformations to reach and anchor RdRp VP1 under the inner capsid shell (Fig. 3D–F).

Of the 5 N termini, that of VP3A<sub>1</sub> (red) attaches to the outside surface of the N-terminal domain of RdRp VP1 (Fig. 3D), while the other 4 interact with the C-terminal bracelet domain (Fig. 3E and F). The N-terminal  $\beta$ -strand of VP3A<sub>3</sub> (yellow) augments 2  $\beta$ -strands ( $\beta$ 1 and  $\beta$ 2 in [SI Appendix, Fig. S4](#)) of the C-terminal bracelet domain to form a 3-stranded antiparallel  $\beta$ -sheet (Fig. 3E), over which the N-terminal region of VP3A<sub>2</sub> (orange) crosses to form another domain-swapped  $\beta$ -sheet (Fig. 3E). The negatively charged residues on the N-terminal loop of VP3A<sub>4</sub> (green), including E19, D21, and D26, interact with the positively charged region (region 3 in Fig. 2I) close to the template exit channel of RdRp VP1 (Fig. 3F). The N-terminal helix



**Fig. 3.** Interactions between BTB RdRp VP1 and the N termini of 5 VP3A molecules, VP3A<sub>1-5</sub>. (A) CryoEM density map of 5 copies of VP3 dimers (VP3A+VP3B) and RdRp VP1, viewed from the inside of the inner capsid. (B) Ribbon diagrams of VP3A, VP3B, and RdRp VP1 in the same orientation as in A. VP3A<sub>1-5</sub> conformers are shown in light gray, with their N termini colored as in A. (C) Superposition of 5 VP3 dimers showing different conformations of VP3A N-terminal fragments. (D–F) Zoom-in views of interactions between individual N termini of 5 VP3A and RdRp VP1 from 3 directions as indicated by the eye symbols in B. See also [Movie S2](#).

of VP3A<sub>5</sub> (blue) extends toward the 5-fold axis and inserts between the inner capsid shell and B $\alpha$ 5 (*SI Appendix*, Fig. S4) of the C-terminal bracelet domain of RdRp VP1 to mediate their interactions (Fig. 3F). Notably, the contributing residues and helix length of the 5 N-terminal helices vary according to the relative position between VP3As and RdRp VP1 (*SI Appendix*, Fig. S8). The N-terminal helices of VP3A<sub>3</sub> (yellow) and VP3A<sub>4</sub> (green) are shorter than the others, and their connecting loops were invisible. These differences suggest that helices are partially unwound to generate longer loops to connect with RdRp VP1 (Fig. 3B). Similar to these observations in BTB, *in situ* cryoEM reconstructions of CPV and ARV also reveal that their transcriptional enzyme complexes (RdRp + NTPase) are anchored to the inner capsid shell by the N termini of capsid shell proteins (22, 24), indicating a conserved mechanism of RdRp-capsid assembly among these viruses despite the existence of the NTPase in turreted reoviruses.

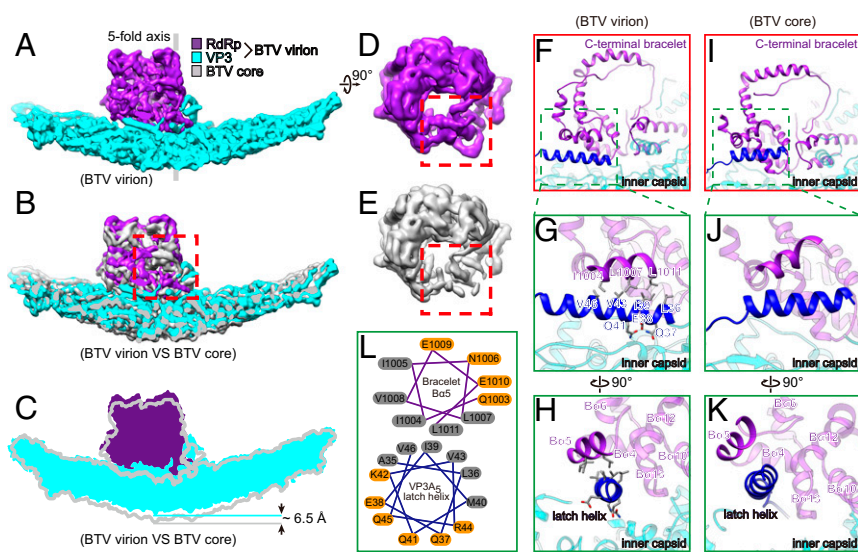
**Detachment of the External Proteins Triggers Conformational Change of RdRp VP1.** Since RdRp VP1 in the BTB virion is incapable of mRNA transcription and becomes functional after the cell entry of the BTB core, we hypothesized that removal of the external proteins might trigger conformational change of RdRp VP1, thus priming it for the synthesis of mRNA. To test this hypothesis, we determined the *in situ* structure of RdRp VP1 inside the BTB core, obtained an asymmetrical subparticle reconstruction of the vertex region at 3.6 Å, and built atomic models of RdRp VP1 and associated N termini of VP3As (*SI Appendix*, Figs. S1, S9, and S10). Superposition of the vertex subparticle reconstructions of the virion and core shows that the inner capsid shell of the core expands through anisotropic movements (with respect to the center of the capsid) of all proteins (Fig. 4A–C and [Movie S3](#)). The maximal movement (~6.5 Å) occurs at the apical domains of the VP3As surrounding the 5-fold axis, to which RdRp VP1s is anchored (Fig. 4C and *SI Appendix*, Fig. S11). Further

comparison between the densities of RdRp VP1 in the virion and the core reveals that the C-terminal bracelet domain undergoes a conformational change, whereas the N-terminal domain and the polymerase core domain remain the same (Fig. 4D and E). The most significant movement of the C-terminal bracelet domain involves the bracelet  $\alpha$  helix B $\alpha$ 5, which shifts over 10 Å along the opposite direction of capsid movement (*SI Appendix*, Fig. S10).

Inserted between the inner capsid shell and the bracelet B $\alpha$ 5 (Fig. 3F), the N-terminal helix of VP3A<sub>5</sub>, which we termed the latch helix, mediates the outward movement of inner capsid shell and local conformational change of the C-terminal bracelet domain during the priming process. Both the latch helix and the bracelet B $\alpha$ 5 are amphipathic helices, and their hydrophobic faces interact with each other, giving rise to a coiled coil structure (Fig. 4G and L). In the virion, the hydrophilic face of the latch helix is attached to polar residues (e.g., R308 and S311) on the inner capsid shell (Fig. 4G and H), stretching the C-terminal bracelet domain to adopt a tensed conformation (Fig. 4F). However, during cell entry, detachment of outer capsid proteins induces the movement of the inner capsid shell and breaks the interaction between the latch helix and inner capsid protein VP3. Then, the latch helix and bracelet B $\alpha$ 5 move together (Fig. 4J and K), changing the C-terminal bracelet domain to a relaxed conformation (Fig. 4I and [Movie S3](#)). The energy released in these processes might fuel the mechanical movement accompanying the initiation of mRNA transcription, and the increased volume inside the capsid would facilitate sliding of genomic dsRNA duplexes.

## Discussion

In this study, we have determined the structure of BTB RdRp by cryoEM and asymmetric, subparticle reconstruction. This structure and other known RdRp structures, including those in CPV (22, 23), MRV (32), ARV (24, 25), and rotavirus (26, 34, 35), share a similar 4-channel architecture of RdRp with catalytic



**Fig. 4.** Structural comparison of the vertex regions in BTV virion and BTV core showing global protein movements and local conformational changes. (A) CryoEM density of RdRp VP1 and inner capsid shell of BTV virion. (B) Superposition of RdRp VP1 and inner capsid shell between BTV core (gray) and BTV virion (colored by protein as in Fig. 1B). Both maps are lowpass-filtered to 6 Å and aligned to their corresponding icosahedral reconstructions as reference, before superposition. See also [Movie S3](#). (C) Schematic illustration of B with the proteins in BTV core shown as gray outline. (D–E) Comparison of RdRp VP1 densities in BTV virion (D) and BTV core (E). Densities of the C-terminal bracelet domain in 2 maps are marked with red boxes. (F–K) Ribbon diagram of RdRp VP1 (purple) and the inner capsid protein VP3 (cyan) in BTV virion (F–H) and BTV core (I–K). Bx<sub>5</sub> (purple) in the C-terminal bracelet domain of RdRp VP1 and the N-terminal helix of VP3A<sub>5</sub> (blue, latch helix) are highlighted in G, H, J, and K. (L) Helical wheel representation of bracelet Bx<sub>5</sub> and the latch helix of VP3A<sub>5</sub> at a view similar to that of (H). Hydrophobic and polar residues are colored in gray and orange, respectively.

site buried in the cavity ([SI Appendix, Fig. S5](#)). However, BTV RdRp has 2 extra motifs, a fingernail and a helix bundle, that have not been observed in other RdRp structures. These 2 motifs are located near the template entry channel, making the channel deeper. The presence of the fingernail in BTV RdRp pushes the terminal RNA to approach the template entry channel in a slightly different orientation. The RdRp in turreted viruses, including CPV, MRV, and ARV, contains 2  $\beta$  hairpins at the bottom part of the fingers subdomain ([SI Appendix, Fig. S5](#)). These  $\beta$  hairpins interact with an NTPase bound to the RdRp in the turreted viruses, but are missing in nonturreted viruses (e.g., BTV and rotavirus), which lack a bound NTPase, suggesting a correlation between the existence of  $\beta$  hairpins and the existence of a bound NTPase.

Viral transcription is highly regulated, as demonstrated biochemically and structurally in viruses in the family of *Reoviridae* (3, 36–40). In these viruses, mRNA transcription is activated by external actions, such as binding S-adenosyl-L-methionine in the single-layered CPV (3, 37, 38) and removing outer shell proteins in the multilayered reoviruses (36, 39, 40). However, how the removal of the outer capsid proteins triggers the initiation of transcription inside the capsid in multilayered reoviruses remains hitherto unknown. Although obvious differences exist in the buffer conditions of the current study and the native cytoplasmic environment encountered by transcriptionally active BTV during infection, our asymmetric reconstructions of the vertex regions of both triple-layered BTV virion and double-layered BTV core particles give us some indications of how the transcription signal is relayed from outside to inside.

Structural comparison between RdRp VP1 and associated inner capsid proteins in both BTV virion and BTV core particles suggests that the inner capsid shell protein VP3A plays an important role in signal transduction. During viral entry, the removal of outer capsid proteins changes the curvature of the inner capsid shell, inducing an outward movement of inner capsid proteins VP3 around the 5-fold axis. This global movement breaks the interaction between the N-terminal helix of VP3A<sub>5</sub> (the latch helix) and inner capsid shell, resulting in the local conformational change of the C-terminal bracelet domain of RdRp VP1. The energy released in these processes and volume enlargement inside the capsid might provide energy and space for the initiation of mRNA transcription. This signal-transduction mechanism from outside to inside might be shared by other members of multilayered reoviruses.

BTV infection of livestock can have significant economic and agricultural impacts on the farming industry (41, 42). Current countermeasures focus on developing life-attenuated live viruses as vaccines. As RdRp is an essential enzyme for viral replication, and transcription not shared by the host, it serves as a promising drug target. Indeed, the publication of X-ray structure of rotavirus RdRp has generated an impetus to structure-based drug design efforts in recent years (26, 43). The atomic structures of BTV RdRp presented here now open the door to design antivirals for therapeutic intervention against bluetongue virus infections.

## Materials and Methods

**Sample Preparation.** BSR cells were infected with BTV-1 at ~0.03 infectious units per cell and maintained at 35 °C for 2 more days postinfection. Infected cells were lysed in Tris buffer (100 mM Tris-HCl at pH 8.8, 50 mM NaCl, 10 mM EDTA) and 0.2% vol/vol Nonidet P-40 at 4 °C for 10 min. The lysate was centrifuged at 2,000g for 10 min to pellet nuclei. The supernatant was incubated at room temperature for 30 to 45 min after adding sodium deoxycholate to the final concentration of 0.1%. After the incubation, the supernatant was loaded onto a double cushion (4 mL 66% wt/wt sucrose and 10 mL 40% wt/vol sucrose in 20 mM Tris-HCl at pH 8.8, 0.2% vol/vol Nonidet P-40) and centrifuged at 100,000g for 1 h at 4 °C. Materials at the interface between the 2 sucrose cushions were collected, diluted 10-fold in 50 mM Tris-HCl buffer at pH 8.8 with 0.2% Nonidet P-40, and incubated in water bath at 35 °C for 45 min after adding *N*-lauroylsarcosine sodium salt to the final concentration of 0.1%. After the incubation, the material was subjected to centrifugation at 16,000g for 10 min at 10 °C to remove aggregates. Then the virion-containing supernatant was centrifuged at 80,000g for 1 h at 10 °C. The resulting pellet consisting of mostly virions and some core particles was resuspended in 20  $\mu$ L of 50 mM Tris-HCl at pH 8.8.

**CryoEM Imaging and Data Processing.** To prepare cryoEM grids, 2.5- $\mu$ L aliquots of sample were applied to thin continuous carbon film on lacey grid, blotted with filter paper, and plunged into liquid ethane. The viruses were imaged with a Titan Krios 300 kV electron microscope equipped with a Gatan K2 Summit direct electron detector at a calibrated magnification of 36,764 $\times$  (pixel size 0.68 Å). Dose-fractionated frames were 2 $\times$  binned (pixel size, 1.36 Å) and aligned for beam-induced drift correction using UCSF MotionCorr2 (44). The defocus values of the micrographs were determined by CTFFIND4 (45) to be in the range of  $-1.1 \mu$ m and  $-3.7 \mu$ m.

The data processing workflow for the reconstruction of RdRp VP1 in BTV core particles is summarized in [SI Appendix, Fig. S9](#). Briefly, the orientation and center parameters of each of the 27,951 high-quality BTV core particles were determined by RELION 2.1 (46) and used to guide the extraction of vertex subparticles with a published procedure (30, 31). After further refinement, each vertex subparticle was expanded with C5 symmetry to get 5 related duplicates, and exhaustive 3D classification was conducted without

applying any symmetry. Five classes with clear secondary structure elements for RdRp VP1 were resolved. We selected vertex subparticles in 1 of the 5 asymmetric classes and removed duplications, resulting in 211,980 good vertex subparticles. Those subparticle images were subjected to another round of 3D classification to further improve the density of RdRp VP1. Finally, 150,346 good vertex subparticles (44.8% of all vertex subparticles) were selected and used for local refinement in RELION. A 3.6-Å-resolution asymmetric cryoEM map of the vertex region of BTV core was obtained and selected as the final map for subsequent model building. The resolution of the cryoEM maps were estimated on the basis of the gold-standard FSC = 0.143 criterion (47). We followed the same icosahedral and subparticle reconstruction procedures to process the 2 datasets of BTV virion independently (*SI Appendix, Fig. S2*). Selected good vertex subparticles from 2 datasets were combined together in the final step of local refinement. A total of 244,063 vertex subparticles were used to obtain asymmetric reconstruction of the vertex region of BTV virion at an average resolution of 3.7 Å.

Please refer to *SI Appendix* for more detailed description of cryoEM imaging and data processing.

**Atomic Modeling, Model Refinement, and Graphics Visualization.** The atomic models of BTV RdRp VP1 and the previously unmodeled N-terminal fragment of capsid shell protein VP3A<sub>1–5</sub> were built de novo with Coot (48) and refined with PHENIX (49) by following the procedure detailed previously (50). Visualization of the atomic model, including figures and movies, are made by UCSF Chimera (51).

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