Supporting Information

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SI Materials and Methods

Plasmids and DNA Templates. To generate the T7 transcripts for in vitro translations, Bluetongue virus 10 (BTV10)-derived plasmids that are available in the laboratory (1, 2) possessing the coding region for each protein were used. The DNA templates for VP1 and VP4 RNAs synthesis were obtained by PCR (KOD polymerase; Novagen), gel-extracted (Promega), precipitated, and resuspended in 15 μL of DEPC-treated water. For VP3, the BTV10 coding sequence was cloned in pcDNA3.1 using primers described in Table S1. The DNA templates for synthesis of VP3, VP6, and VP7 RNAs were obtained by NotI digestion of the plasmids, followed by phenol/cholorophorm extraction, precipitation, and resuspension in 15 μL of DEPC-treated water. For generation of the 10 BTV1 uncapped ssRNA segments, exact-copy vectors were used as templates (3). The synthetic RNAs were generated without cap analog to avoid the translation during the assembly assay as well as during the infection.

Generation of T7 Transcripts. The capped RNAs coding for VP1, VP3, VP4, and VP6 were generated using MessageMachine kit (Ambion) according to the manufacturer's instructions. The T7 reaction products were phenol/cholorophorm-extracted, purified using G25 columns (GE Healthcare), precipitated, and resuspended in 15 μL of DEPC-treated water. The 10 uncapped BTV1 ssRNA were generated using T7 Ribomax kit (Promega) according to the manufacturer's instructions and purified, as described above. RNA integrity was verified by electrophoresis on 1% agarose in Mops (morpholinepropanesulfonic acid) buffer in the presence of formaldehyde, using standard techniques.

End-Labeling of ssRNA. For end-labeling of ssRNA, 500 ng of each BTV uncapped ssRNAs were labeled at the 3′ end, in the presence of 0.2 μCi/ μL ^{32}P-pCp (Amersham Bioscience) in a final volume of 15 μL, using RNA T4 ligase (Fermentas) according to the manufacturer's recommendations. The reaction was incubated overnight at 4 °C and stopped by phenol/chlorophorm extraction, precipitated, and resuspended in 5 μL of RNase-free water. The same protocol was used for labeling the marker ssRNAs (Promega), using 1 μL of RNA (1 μg). Samples were analyzed by 9% native-PAGE, dried, and exposed to Storage Phosphor screen (GE Healthcare).

Protein Detection. SDS-PAGE and Western blots were performed to monitor the protein synthesis, using standard techniques. Briefly, 1:6,000 dilutions of polyclonal mouse anti-VP4 antibody or rabbit anti-VP1, anti-VP3, anti-VP6, and anti-VP7, together with 1:10,000 dilution of anti-mouse or anti-rabbit coupled to peroxidase secondary antibodies (Sigma) were used. Detection was performed using SuperSignal West Femto ECL kit (Pierce) and autoradiography.

For the detection of RNA-protein complexes, Bis-Tris 3% to 12% gradient native gels (Invitrogen) were used according to the manufacturer's instructions, followed by silver staining, as described by Gromova and Celis (4).

Cell Culture and Infections. KC cells were grown at 28 °C in Schneider medium (Gibco) supplemented with 5% FCS (Gibco).

For infection with the reconstituted particles, sucrose gradient fractions were diluted in Schneider medium (1:3) lacking FCS and added directly to KC cells monolayer (seeded in six-well plates at 2×10^6 per well); after adsorption for 1 h at room temperature, 2 mL of Schneider medium plus 5% FCS were added and incubated at 28 °C. At 6 d postinfection, cells were either immunostained for BTV protein detection or harvested and stored at 4 °C. The putative virus stock was amplified in KC cells by a further four passages and dsRNAs were extracted from the fourth passage.

Double-Stranded RNA Extraction. KC cells were harvested and treated with Tri-reagent (Invitrogen) following the manufacturer's instructions. After the extraction, ssRNAs were precipitated with 8 M LiCl (Sigma) overnight at 4 °C. The supernatant containing dsRNAs was precipitated with 1 volume of isopropanol (Sigma) and glycogen (Fermentas), and resuspended in 5 μL of RNase-free water. Samples were analyzed by 9% native-PAGE followed by ethidium bromide staining.

Immunofluorescence Staining. Infected cells after 6 d postinfection were fixed with 4% PFA in PBS. Staining was performed using either BTV1 anti-NS1 or anti-NS2 rabbit antiserum followed by Alexa Fluor 488 conjugated anti-rabbit (Sigma). VP5 was detected using a guinea pig antiserum raised against BTV1-VP5, followed by Alexa Fluor 566 conjugated anti-guinea pig (Sigma). Nuclear staining was achieved by using Hoescht 33342 at 3.3 μg/mL in the secondary antibody solution. Digital images were recorded with an LSM510 microscope (Carl Zeiss) using a 63× oil objective. Images were processed with the LSM image browser software (Carl Zeiss).

Electron Microscopy. Carbon-coated copper 400 mesh electron microscopy grids were prepared with 1% Alcian blue and washed with water and 10 μL aliquot of the fraction containing putative cores were adsorbed onto the grids. After 15 min, the grids were washed with water and negatively stained with 2% (wt/vol) uranyl acetate. The grids were examined under a JEOL 100CX electron microscope at 80 kV.

1. French TJ, Roy P (1990) Synthesis of bluetongue virus (BTV) corelike particles by a recombinant baculovirus expressing the two major structural core proteins of BTV. *J Virol* 64:1530–1536.
2. Loudon PT, Roy P (1991) Assembly of five bluetongue virus proteins expressed by recombinant baculoviruses: Inclusion of the largest protein VP1 in the core and virus-like proteins. *Virology* 180:798–802.
3. Boyce M, Celma CC, Roy P (2008) Development of reverse genetics systems for bluetongue virus: Recovery of infectious virus from synthetic RNA transcripts. *J Virol* 82:8339–8348.
4. Gromova I, Celis JE (2006) *Protein Detection in Gels by Silver Staining: A Procedure Compatible with Mass-Spectrometry in Cell Biology: A Laboratory Handbook* (Elsevier. Academic Press, San Diego), 3rd Ed.

Fig. S1. In vitro translation of BTV10 VP1, VP3, VP4, VP6, and VP7. The five proteins were expressed by in vitro translation system using wheat germ extracts, detected by Western blot using specific polyclonal antibodies for each protein, and developed by ECL. Lane1, VP1; lane 2, VP3; lane 3, VP4; lane 4, VP6; lane 5, VP7.

Fig. S2. Intermediates of subcore assembly pathway. Different combinations of subcore proteins (two, three, or four) were used in assembly assay, products were fractionated on sucrose gradients, and analyzed by native-PAGE followed by silver staining. The relevant and representative fraction for each combination is shown and indicated. The two *Right* panels are from the same gel, which is different from the *Left* panel gel. The marker was included in each gel (M).

Fig. S3. Assembly assay in the absence of a complete set of ssRNAs. (*A*) The assembly assay was tested in the presence of nine segments (^{32}P-labeled), excluding S10, and analyzed by native gel followed by silver staining. (*B*) The assembly assay was tested in the absence of the 10 ssRNAs and analyzed by native gel followed by silver staining. (*C*) Fraction 6 from the assembly assay (*A*) was analyzed for packaging capacity. Lane M: synthetic BTV1 ssRNAs ^{32}P-labeled (as described in *Materials and Methods*), lane (–) S10: ssRNAs extracted from fraction 6 shown in *A* and analyzed by denaturing agarose gel and exposed to the Storage Phosphor screen.

Table S1. Primers used

Gene	Sequence
pBTV10_VP3codreg_NotIrev	5′ GCTTAATTGCGGCCGCCTACACAGTTGGCGCAGCCAGCTTGG 3′
pBTV10_VP3codreg_fw	5′ AAACCCGGGATGGCTGCTCAGAATG 3′
BTV10-VP1_T7pr-fw	5′ GGGCCCTAATACGACTCACTATAGGGTTTCCACCATGGTCGCAATCACCGTGCAAGGTGCACAGCTC 3′
BTV10-VP1_polyA-rev	5′ 5′ TTTTTTTTTTTTTTTTTTTTCAAACGAATTGGTTCTCAAGATTTCTAACTGTCATTCTATTGGATCGATCAACTTC 3′
BTV10-VP4_T7pr-fw	5′ GGGCCCTAATACGACTCACTATAGGGTTTCCACCATGCCTGAGCCACACGCAGTACTCTATGTCACA 3′
BTV10-VP4_polyA-rev	5′ TTTTTTTTTTTTTTTTTTTTTATAAGTGACGCAGTAATTCTAGCCACGAATCAATCGAGGCTCCCTC 3′