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An icosahedral algal virus has a complex unique vertex decorated by a spike

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Author contributions: M.V.C., Y.A.K., C.X., and M.G.R. designed research; M.V.C. and Y.A.K. performed research; Y.A.K., V.D.B., A.J.B., X.Y., P.C.R., T.S.B., and J.L.V.E. contributed new reagents/analytic tools; M.V.C. and M.G.R. analyzed data; and M.V.C. and M.G.R. wrote the paper.

Abstract — *Paramecium bursaria Chlorella* virus-1 (PBCV-1) is an icosahedrally shaped, 1,900-Å-diameter virus that infects unicellular eukaryotic green algae. A 5-fold symmetric, 3D reconstruction using cryo-electron microscopy images has now shown that the quasispherical virus has a unique vertex, with a pocket on the inside and a spike structure on the outside of the capsid. The pocket might contain enzymes for use in the initial stages of infection. The unique vertex consists of virally coded proteins, some of which have been identified. Comparison of shape, size, and location of the spike with similar features in bacteriophages T4 and P22 suggests that the spike might be a cell-puncturing enzyme. The pentameric vertices may have been missed in previous analyses of many other viruses that had been assumed to be perfectly icosahedral.

Keywords — 5-fold averaging, cryo-electron microscopy reconstruction, *Paramecium bursaria Chlorella* virus-1, cell entry, specialized vertex protein

*Paramecium bursaria Chlorella* virus-1 (PBCV-1) belongs to the genus *Chlorevirus* of the Phycodnaviridae family. Together with iridoviruses, asfarviruses, and mimiviruses, they belong to a group of nucleocytoplasmic large DNA viruses that may have evolved from a common ancestor, predating the divergence of prokaryotes and eukaryotes (1–4). PBCV-1 infects the unicellular eukaryotic green alga *Chlorella* NC64A by attaching rapidly and irreversibly to the algal cell wall (2, 5). Attachment appears to occur at one of the vertices and might involve hair-like appendages (3). After digestion of the cell wall by virion-packaged enzymes in the vicinity of the point of attachment, the internal viral membrane fuses with the host membrane. Viral DNA and some associated proteins then enter into the cell, leaving the empty capsid on the cell surface (3). PBCV-1 has a multilayered structure, with an outer glycoprotein capsid that surrounds a lipid bilayer membrane and a ds-DNA genome (4, 6). The 331-kbp PBCV-1 genome codes for 11 tRNAs and ≈365 putative proteins (7), of which more than 100 are present in the mature virion. Vp54, a glycoprotein and the major capsid protein, represents about 40% by weight of the total protein content in the virus. The crystal structure of PBCV-1 Vp54 had been fitted into the 26-Å-resolution cryoEM density map (6, 10) to generate a pseudoatomic structure of the capsid. However, the 5 jelly rolls surrounding each pentameric vertex form a special capsomer in which, presumably, the monomers contain only 1 jelly roll. Thus, as in the adenovirus structure (11), the capsid protein at the virus vertices differs from the major capsid protein, Vp54.

Results and Discussion

**PBCV-1 Has a Unique Vertex Topped by a Spike-like Structure.** Unprocessed cryoEM images of PBCV-1 usually indicate that the virions have a dense external capsid surrounding a membrane-like feature that, in turn, surrounds a nucleocapsid core. In some images, there is a gap between the enveloped nucleocapsid core and the surrounding capsid at one of the icosahedral pentameric vertices (Figure S1). A similar gap occurs in mimivirus particles, which also have a unique pentameric vertex (12). Previous reconstructions of PBCV-1 (4, 6) did not show a unique vertex, because of the icosahedral averaging process. Hence, the cryoEM images of PBCV-1 have now been reanalyzed by assuming only the presence of 5-fold symmetry about an axis passing through the center of one selected vertex (see Materials and Methods). The resulting reconstruction clearly showed the capsid with the previously described trisymmetric pentamers and pentasymmetric structures (Figure 1A) and the associated lipid membrane (Figure 1B). However, 1 of the 2 vertices on the enforced 5-fold axis had a 250-Å-long and 50-Å-wide cylindrical spike (Figure 1C). This feature can be recognized in selected particles where the spike is in the plane of the micrograph (Figure S1) (3, 13). The possibility that PBCV-1 might have a special vertex had been predicted earlier in a paper that describes the unique vertex of the dsDNA bacteriophage PRD1 (14).

Recognition of the unique vertex of PBCV-1 had been missed for many years on account of assuming icosahedral symmetry in structural analyses (4, 10). After the discovery of a unique vertex for herpes simplex virus (HSV) (15) and mimivirus (12, 16), to the best of our knowledge, PBCV-1 is the third icosahedral, eukaryotic virus that has been recognized to have a unique vertex. It would therefore be possible that many more icosahedral eukaryotic viruses may require unique vertices for the successful execution of their life cycle. However, the complex structure of unique vertices as occurs in most tailed bacte-
The Unique Vertex Contains Special Capsomers. As mentioned above, the capsomers on 11 of the 12 vertices consist, presumably, of 5 single jelly-roll monomers. However, the pentasymmetron at the unique vertex (Figure 2A) has a different density distribution for the pentamic capsomer than those at the 11 other vertices (Figure 2B). The pentamic capsomer at the unique vertex binds the spike and is surrounded by 5 pentameric capsomers. The peripentonal capsomers (no. 1 capsomers in Figure 2A) have additional density on their surfaces compared with the major capsomers (Vp54 trimers; no. 7 and no. 8 capsomers in Figure 2A). The 5 peripentonal capsomers at the unique vertex are surrounded by 10 capsomers (no. 2 and no. 3 capsomers in Figure 2A) that are alternatively similar to the major capsomers and the peripentonal capsomers. These, in turn, are surrounded by 15 capsomers that form the edge of the pentasymmetron. Of these, 10 resemble the major capsomer (no. 4 and no. 6 capsomers in Figure 2A), and the other 5 (no. 5 capsomers in Figure 2A) are different (Table S1).

Large insertions in jelly-roll structures occur invariably between the D and E and between the F and G β-strands in either the first or second jelly-roll fold within the capsid proteins of the nucleocytoplasmic large DNA viruses (Figure S2). Thus, the additional density in the peripentonal capsomers might correspond to an insertion at one of these loops. BLAST analysis (29) of the 6 PBCV-1-encoded proteins with sequence similarity to Vp54 showed that only the gene product of ORF A622L is larger than Vp54 and has an insertion of 111 residues between β-strands D and E in the first jelly roll of the sequence. This number of amino acids occupies a predicted volume of ~15,000 Å³ (calculated by using the peptide property calculator of A. Chazan, available at www.basic.northwestern.edu/biotools/proteincalc.html), which agrees with the ~15,500 Å³ extra density of the peripentonal capsomers in comparison with Vp54.

The Virus Has a Partially Empty Pocket. Within the virion, near the pentameric capsomer at the unique vertex, is an additional density (150 Å wide and 100 Å long) that is similar in size and location to the portal proteins in tailed phages, such as in phi29 (30), SPF1 (31), and P22 (Figure 3A) (32, 33). The PBCV-1 portal-like protein is attached to the peripentonal capsomers and not to the special pentameric capsomer. The portal protein in bacteriophages is a dodecamer. If PBCV-1 also has a 12-fold symmetric portal, then the 5-fold averaging used in the cryoEM reconstruction would blur the boundaries between subunits. Although the role of the PBCV-1 portal-like protein is unclear, in tailed phages the portal protein is probably involved in both DNA packaging and DNA ejection (7). As reported previously (4), an internal lipid bilayer envelopes the PBCV-1 nucleocapsid. In previousicosahedral re-
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constructions of PBCV-1, the lipid membrane follows the contour of the capsid. However, the present 5-fold averaged map shows that the lipid membrane departs from icosahedral symmetry in the region near the unique vertex, creating a partially empty pocket between the 5 icosahedral faces surrounding the unique vertex and the membrane (Figure 1C). The pocket measures ≈325 Å along a radial direction at its widest point. The average density in the pocket is substantially less than that within the nucleocapsid. Cell wall-degrading enzymes are thought to be stored near the unique vertices of tailed bacteriophage (32–34). Thus, the pocket in PBCV-1 may function as a reservoir for enzymes and other proteins that might be excreted through the unique vertex to digest the cell wall of the chlorella cells in the initial stages of infection (3).

Apart from the general disordered distribution of density within the pocket, a set of well-ordered features line the inside of the viral capsid within the pocket. These features are similar to a class of minor capsid proteins ("finger proteins") observed in the $T = 147$ Chilo iridescent virus (CIV) (35). The PBCV-1 finger proteins have 2 distinct globular domains (Figure 3B): one is as-

Figure 2. Stereodagrams of the pentasymmetrons at (A) the unique vertex and (B) the 5-fold axis opposite the unique vertex. Note the difference in the density distribution of the pentameric capsomer (red) in A and B. The top end of the narrow spike can also be seen in A. Although all of the major capsomers (Vp54 trimers) surrounding the pentasymmetrons and in the pentasymmetron shown in B have very similar density distributions, this is not the case for the pentasymmetron at the special vertex shown in A. The peripentonal capsomers around the unique vertex are shown in gold and have an extended amino acid insertion. The 10 capsomers surrounding the peripentonal capsomers are colored blue and green. Whereas the green capsomers are similar to the peripentonal capsomers, the blue capsomers are similar to the major capsomers. The capsomers in the outer pentasymmetron ring are colored yellow, dark blue, and pink. The yellow and pink capsomers are similar in structure to the major capsid protein, whereas the dark blue capsomers have a slightly different density distribution.

Figure 3. View of features inside the PBCV-1 capsid. All panels are colored as in Figure 1C. (A) The portal within the capsid at the unique vertex. (B) View of the unique vertex from the inside of the virus. Note the finger proteins at a slightly smaller radius appear red. No finger proteins are associated with the pentasymmetron at the unique vertex. (C) View of the vertex opposite the unique vertex. Note the lack of finger protein features and the low-density gap corresponding to the lipid bilayer membrane roughly in the yellow density.
sociated with the center of an external capsomer, and the other is associated with the gap between 3 neighboring capsomers (Figure 4). However, in PBCV-1, unlike in CIV, the cryoEM data were only 5-fold-averaged, thereby making it possible to differentiate between finger proteins associated with trisymmetrons around the unique vertex (Figure 3B) and the absence of finger proteins in the other trisymmetrons (Figure 3C). It may be that finger proteins prevent the lipid membrane from attaching to the inside of the viral capsid in the vicinity of the unique vertex, thus facilitating the formation of the pocket that contains the molecular “soup” necessary for infection.

The Fibered Capsomers Have a Unique Symmetry. A previously published PBCV-1 cryoEM reconstruction (36) showed that 1 capsomer per icosahedral asymmetric unit (3 per trisymmetron) has a protruding fiber. However, the 5-fold-averaged reconstruction reported here shows that none of the 20 trisymmetrons have all 3 symmetry-related fibers (Figure S3). Furthermore, some trisymmetrons have an additional type of fibered capsomer (Figure S4). These fibers might be involved in attaching and orienting the virus to the host during infection.

Conclusions
The major capsid proteins of PRD1, Sulfolobus turreted icosahedral virus, and PBCV-1, infecting prokaryotes, archaea, and eukaryotes, have a “double jelly-roll” fold, suggesting that at least as far as their capsids are concerned, they may have had a common precursor (6, 37–40). Similarly, many or most tailed dsDNA bacteriophages (41, 42) and HSVs (43) use the “HK97” fold for the structure of their major capsid protein, suggesting a common ancestry for these viruses. Yet, it is shown here that there might be some structural and functional relationship between the cell-puncturing device of bacteriophage T4 and the spike structure of PBCV-1, which could indicate perhaps convergent evolution for the development of a mechanical device for penetrating the external cell defenses for viruses that have quite different origins for their major capsid proteins.

The results also demonstrate that global enforcement of icosahedral symmetry in viruses can obscure vital information about the structural properties of virions. Indeed, transient slight deviation from icosahedral symmetry may be as important to the viral life cycle (23, 44) as precise icosahedral symmetry is for viral assembly. Larger viruses, such as PBCV-1, PRD1, HSV, mimivirus, or T4, have the capacity to code for special proteins associated with a special vertex, as is witnessed by the presence of genes that are homologues of the major capsid protein and might be required for spike or tail formation. However, smaller viruses that do not have specialized genes might also have special vertices (25) that are associated with the ends of the genome that are stored for exit when infection is initiated. Nevertheless, the presence of unique features required for cell entry might not be necessary for all viruses. Whereas some viruses, such as PBCV-1 and tailed bacteriophages, remain outside the cell on infection, inserting only their genome through the cell wall or plasma membrane, other viruses merely become unstable within the acid pH of endosomes. Thus, it is likely that many or even most virus structures reported to date might be reexamined to establish the presence of special nonsymmetric features, such as special vertices and attachment fibers.

Materials and Methods
PBCV-1 samples were prepared for cryoEM as described previously (4). Micrographs of frozen-hydrated samples were recorded on Kodak SO-163 film in CM200 and CM300 FEG transmission electron microscopes (Philips). Micrographs were digitized at 7-Å intervals, and the resulting data were interpolated to yield pixels corresponding to 8.0 Å at the specimen. The program RobEM (45) was used to select 5,149 particles from 228 micrographs and to determine their defocus levels by fitting the contrast-transfer functions to the Fourier transforms averaged incoherently over all particle images for each micrograph. The 3D reconstruction was computed by using images that had only been corrected for phase reversals. A similar procedure for producing a 5-fold average density map, described previously (44), was developed by integrating functions with the SPIDER program (46) and a modified version of XMIPP (47). The first stage of the procedure assumed icosahedral symmetry to determine the center position and relative orientation of each re-
corded particle. The second stage of the procedure assumed only 5-fold symmetry by selecting a unique orientation from the 12 icosahedrally related orientations with respect to an increasingly more asymmetric model as refinement proceeded. The test for selecting the unique orientation from the 12 possible orientations was based on comparing only the density in the vicinity of the unique vertex with each particle projection. Various limits were tried for defining the size and shape of the mask that defined the volume around the unique vertex. After 10 cycles, the choice of orientation of each boxed image had mostly converged. The final resolution of the cryoEM reconstruction was 22Å, determined as the point at which the Fourier shell correlation coefficient between 2 half-datasets fell below 0.5.

Figures were created by using the program CHIMERA (48).

Supplementary Material
Supporting Information for this article (Figures S1–S4 and Table S1) follows the References.

Acknowledgments
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The EM density map reported in this paper has been deposited in the EM Databank, http://emdatabank.org (accession no. EMD-1597).

References
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Figure S1. CryoEM micrograph of a PBCV-1 particle showing a pocket near the 5-fold vertex marked with a black asterisk (*). (Scale bar = 500 Å.)
Figure S2. Diagrammatic representation of the first (green) and second (red) jelly-roll structures within the major capsid proteins of viruses PBCV-1, PRD1, adenovirus, and CIV. The β-strands along the polypeptides are labeled B through I. Amino acid numbers are given at the beginning and end of the β-strands. Note that larger insertions are restricted to the loops between D and E as well as F and G.
Figure S1. Diagrammatic representation of the 4 layers of trisymmetrons within the virus viewed in a direction perpendicular to the unique S-fold axis. The remaining trisymmetrons would be generated by a further 4 successive rotations about the common S-fold axis. One type 1 fibered capsomer (Figure S4A) occurs in all trisymmetrons in a position that is consistent with icosahedral symmetry. However, the symmetry at level d has 2 symmetry-related, type 1 fibered capsomers. The type 2 fibered particles (Figure S4B) occur only in the level b trisymmetrons.
**Figure S4.** Stereodiagrams of fibered capsomer cryoEM densities. (A) Type 1 fibered capsomers present in quasiicosahedrally related positions at least once in each trisymmetron. (B) Type 2 fibered capsomer present only in level b trisymmetrons (see Figure S3). Contrary to type 1 fibered capsomers, these capsomers have density on their central 3-fold axes.

**Table S1.** Correlation between the cryoEM densities of the capsomers surrounding the unique vertex

<table>
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<tr>
<th>Capsomer</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>Peripental</th>
<th>Layer adjacent to peripental</th>
<th>Outside layer of pentasymmetron</th>
<th>Major Vp54 capsomers in trisymmetron</th>
</tr>
</thead>
<tbody>
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<td>1</td>
<td>1.000</td>
<td>0.816</td>
<td>0.900</td>
<td>0.795</td>
<td>0.788</td>
<td>0.787</td>
<td>0.799</td>
<td>0.797</td>
<td>Peripental</td>
<td>Layer adjacent to peripental</td>
<td>Outside layer of pentasymmetron</td>
<td>Major Vp54 capsomers in trisymmetron</td>
</tr>
<tr>
<td>2</td>
<td>1.000</td>
<td>0.800</td>
<td>0.895</td>
<td>0.805</td>
<td>0.870</td>
<td>0.889</td>
<td>0.869</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
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<td>0.812</td>
<td>0.832</td>
<td>0.851</td>
<td>0.822</td>
<td>0.827</td>
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<td>4</td>
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<td>0.815</td>
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<td>0.940</td>
<td>0.914</td>
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<tr>
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<td>0.836</td>
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<td>0.940</td>
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<td>7</td>
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Five no. 1 (peripental) capsomers surround the pentameric capsomer at the vertex. These are surrounded by 5 no. 2 and 5 no. 3 type capsomers. Of these no. 2 is most similar to the major Vp54 trimeric capsomers represented here by no. 7 and no. 8 (yellow), whereas capsomer no. 3 is similar to the peripental capsomer no. 1 (red). The outside capsomers of the pentasymmetron is composed of capsomers no. 4, 5 and 6. Of these capsomers no. 4 and no. 6 correlate with the major capsomer. Capsomer no. 5 looks like a mixture of the peripental capsomer no. 1 and the major capsomers no. 7 and no. 8 found in the trisymmetrons. These correlations correspond to the visual inspection of the capsomers shown in Fig 2A.