Structural Characterization of H-1 Parvovirus: Comparison of Infectious Virions to Empty Capsids

Sujata Halder,^a Hyun-Joo Nam,^{a*} Lakshmanan Govindasamy,^a Michèle Vogel,^{b*} Christiane Dinsart,^b Nathalie Salomé,^b Robert McKenna,^a Mavis Agbandje-McKenna^a

Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, Florida, USA^a; Research Program Infection and Cancer, Division of Tumor Virology, DKFZ F010–Inserm U701, Deutsches Krebsforschungszentrum, Heidelberg, Germany^b

The structure of single-stranded DNA (ssDNA) packaging H-1 parvovirus (H-1PV), which is being developed as an antitumor gene delivery vector, has been determined for wild-type (wt) virions and noninfectious (empty) capsids to 2.7- and 3.2-Å resolution, respectively, using X-ray crystallography. The capsid viral protein (VP) structure consists of an α -helix and an eight-stranded anti-parallel β -barrel with large loop regions between the strands. The β -barrel and loops form the capsid core and surface, respectively. In the wt structure, 600 nucleotides are ordered in an interior DNA binding pocket of the capsid. This accounts for ~12% of the H-1PV genome. The wt structure is identical to the empty capsid structure, except for side chain conformation variations at the nucleotide binding pocket. Comparison of the H-1PV nucleotides to those observed in canine parvovirus and minute virus of mice, two members of the genus *Parvovirus*, showed both similarity in structure and analogous interactions. This observation suggests a functional role, such as in capsid stability and/or ssDNA genome recognition for encapsulation. The VP structure differs from those of other parvoviruses in surface loop regions that control receptor binding, tissue tropism, pathogenicity, and antibody recognition, including VP sequences reported to determine tumor cell tropism for oncotropic rodent parvoviruses. These structures of H-1PV provide insight into structural features that dictate capsid stabilization following genome packaging and three-dimensional information applicable for rational design of tumor-targeted recombinant gene delivery vectors.

H-1 parvovirus (H-1PV) is a member of the rodent subgroup of the *Parvovirus* genus of the single-stranded DNA (ssDNA) *Parvoviridae* (1). H-1PV was first isolated from rats transplanted with HEP-1, a human liver adenocarcinoma cell line (2), and also from aborted human fetuses (3). Recombinant vectors based on H-1PV and a number of other rodent parvoviruses, including minute virus of mice (MVM) and LuIII, are promising candidates for antitumor delivery vectors, particularly for cytoreductive and immunogene therapy approaches (reviewed in references 4 and 5). The inherent oncotropism of these autonomous parvoviruses is based on their dependence on cellular proliferation factors expressed during the S phase and the differentiated state of the host cell (6, 7). The rodent parvoviruses display oncopreferential cytotoxic activity *in vitro* and also possess an oncosuppressive potential, inhibiting the formation of spontaneous and chemical or virus-induced tumors *in vitro* and *in vivo* (8–13). These viruses can also persistently infect their natural hosts, do not integrate their genome into cellular chromosomes, and are not associated with human disease (reviewed in reference 4).

Recombinant rodent parvovirus vectors targeted for tumor therapy utilize a double-edged strategy that takes advantage of their inherent oncotropism and selective cytotoxicity plus their ability to deliver therapeutic genes that code for toxins, such as thymidine kinase, or host immune response enhancers, such as cytokines/chemokines (14–19). The first clinical study of H-1PV in 1965 injected wild-type (wt) virus into two osteosarcoma patients (20). This treatment did not completely ablate tumor development, possibly due to the generation of neutralizing antibodies. In other studies, H-1PV was assessed for the killing of human neuroblastoma and hepatoma cells and demonstrated tumor-selective lytic effects and low toxicity for nontransformed cells (21, 22). Local, intranasal, or systemic treatment of advanced rat and

human gliomas in rat models with H-1PV was also reported to induce regression (23, 24). These promising results set the stage for the first phase I/IIa clinical trial using replication-competent H-1PV in patients with progressive primary or recurrent glioblastoma multiforme (25).

The parvoviruses encapsulate a linear ssDNA genome of ~5 kb, with small terminal palindromes, into a T=1 icosahedral capsid with an overall diameter of ~260 Å. The H-1PV genome encodes two nonstructural proteins (NS; NS1 and NS2) and two capsid viral proteins (VPs; VP1 and VP2), driven by promoters P4 and P38, respectively. NS1 (76 kDa) is a phosphoprotein with helicase, ATPase, DNA-nicking, and sequence-specific DNA binding activities essential for replication, and it is also the major mediator of cytotoxicity (26). NS2 (21 kDa) is also required for virus replication and cytotoxicity (26). The capsid VPs are overlapping in amino acid sequence, with VP1 (81 kDa) and VP2 (65 kDa) produced by alternative splicing from the same mRNA, and are expressed at a ratio of 1:5 (27). VP1 and VP2 have a common C-terminal sequence (593 amino acids [aa]), with VP1 containing a unique N-terminal region of 142 amino acids (VP1u). In wt virions (DNA containing) but not in empty capsids, VP3 is gen-

Received 11 December 2012 Accepted 14 February 2013

Published ahead of print 28 February 2013

Address correspondence to Mavis Agbandje-McKenna, mckenna@ufl.edu.

* Present address: Hyun-Joo Nam, Department of Bioengineering, University of Texas at Dallas, Richardson, Texas, USA; Michèle Vogel, Inserm U692, Laboratoire de Signalisations Moléculaires et Neurodégénérescence, Strasbourg, France.

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doi:10.1128/JVI.03416-12

erated by posttranslational cleavage of 18 or 21 amino acids from the N terminus of VP2 (but not of VP1). VP1 is present at ~10 copies per virion or capsid (of 60 VPs) with ~50 copies of VP2 in empty capsids or a mixture of VP2 and VP3 in virions, with VP3 being the major component.

The structures for several members of the mammalian *Parvoviridae* have been determined using X-ray crystallography and/or cryoelectron microscopy and image reconstruction (cryoreconstruction), including those of MVM, canine parvovirus (CPV), feline panleukopenia virus (FPV), and porcine parvovirus (PPV) (28–36). In most of these structures, only ~520 to 550 residues (depending on the virus) of VP2 (or VP3 in virions) in the overlapping polypeptide region is observed. The only exception is the cryoreconstructed structure of B19V, in which the N-terminal amino acids were also ordered (37). Despite differences in amino acid sequence between family members, the VP structural topology is highly conserved among the parvoviruses (38). Variable capsid surface loops govern many biological functions, including receptor binding, tissue tropism, pathogenicity, and antigenicity, and also form the structural basis of the phenotypic differences among members and between strains of the same virus (39).

Here, we report the structure determination of H-1PV virions and empty capsids using X-ray crystallography to 2.7- and 3.2-Å resolution, respectively. These structures differ only in the side chain conformations for some of the residues that surround nucleotides ordered in a conserved parvovirus DNA binding pocket in the virion structure. This indicates a potential evolutionarily conserved role in capsid DNA packaging and stability. The VP structure conserves the β -barrel and α A helix topology previously reported for other parvoviruses, including CPV, FPV, MVM, and PPV, but exhibits distinctive differences at the apex of the large insertion loops between the β -strands. These differences are located at capsid surface regions and are reported to mediate receptor binding, tumor tropism, and antibody recognition.

MATERIALS AND METHODS

Virus production, purification, crystallization, X-ray diffraction data collection, and data processing. H-1PV virions and empty capsids were produced in NB324K cells, purified by CsCl gradient ultracentrifugation, and crystallized as previously described (40). For the virions, X-ray diffraction data collection on crystals grown in 10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 8 mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, and 3% polyethylene glycol (PEG) 8000, processing of the data, and structure determination to 2.7-Å resolution by the molecular replacement method have also been reported (40). For the empty capsids, a total of 251 usable X-ray diffraction images were collected from a single crystal, grown under conditions similar to those for the virions, at the X29 beamline ($\lambda = 1.0895$ Å) of the National Synchrotron Light Source (NSLS; Brookhaven National Laboratory). The data were collected on an ADSC Quantum 315 charge-coupled device (CCD) detector with a crystal-to-detector distance of 400 mm, an oscillation angle of 0.3°, and exposure time of 20 s per image. The crystals diffracted X rays to ~3.2-Å resolution. The data were indexed and integrated with the HKL2000 suite of programs and scaled and merged with SCALEPACK (41). The data set scaled with an R_{sym} of 11.4% (61.3% completeness) (Table 1). Similar to the virions, the empty capsids crystallized in the $P2_1$ space group with unit cell parameters reported in Table 1. The Matthews coefficient (V_M) was calculated to be $2.9 \text{ Å}^3 \text{ Da}^{-1}$ (assuming a molecular mass of $\sim 4.1 \times 10^6$ Da), corresponding to a solvent content of 58% (42). There are two capsids per unit cell related by a 2_1 screw axis, with one particle occupying a crystallographic asymmetric unit. The data collection

TABLE 1 Data collection, processing, and refinement statistics of H-1PV structures

Parameter	Value(s) ^a for:	
	Empty capsid	Virion ^b
Wavelength (λ ; Å)	1.0895	0.9186
No. of films	251	392
Space group	$P2_1$	$P2_1$
Unit cell parameters		
a, b, c (Å)	255.2, 350.1, 272.1	255.4, 350.4, 271.6
β (°)	90.12	90.34
Resolution (Å)	40–3.2 (3.3–3.2)	50–2.7 (2.8–2.7)
Total no. of reflections	4,903,429	16,673,125
No. of unique reflections	479,478 (45,513)	1,210,268 (123,000)
Crystal mosaicity (°)	0.5–0.7	0.3–0.5
Completeness (%)	61.3 (58.3)	93.1 (94.7)
R_{sym} (%)	11.4 (40.0)	13.2 (37.8)
Redundancy	1.7 (1.5)	2.6 (2.4)
I/σ	4.9 (1.3)	6.9 (2.4)
V_M ($\text{Å}^3/\text{Da}$)	2.9	2.1
No. of atoms (protein/ $\text{H}_2\text{O}/\text{DNA}/\text{ion}/\text{other}$ solvent)	4,366/18/0/2/0	4,385/131/213/5/16
Avg B_{factor} (Å^2) (protein/ $\text{H}_2\text{O}/\text{DNA}/\text{ion}/\text{other}$ solvent)	61.8/44.2/–/40.4/–	30.8/32.8/79.5/33.1/42.5
$R_{\text{factor}}^e/R_{\text{free}}^f$ (%)	25.6/25.6	21.6/21.6
RMSD bonds (Å), angles (°)	0.007, 1.37	0.006, 1.37
Ramachandran statistics (%) (most favored/allowed/outliers)	98.9/1.1/0	98.9/1.1/0

^a Values in parenthesis are for the highest resolution shell.

^b Processing statistics were previously reported in reference 40.

^c $R_{\text{sym}} = (\sum |I - \langle I \rangle| / \sum \langle I \rangle) \times 100$, where I is the intensity of an individual reflection with indices h, k , and l , and $\langle I \rangle$ is the average intensity of all symmetry equivalent measurements of that reflection; the summation is over all intensities.

^d V_M , Matthews's coefficient.

^e $R_{\text{factor}} = (\sum |F_o| - |F_c| / \sum |F_o|) \times 100$, where F_o and F_c are the observed and calculated structure factor amplitudes, respectively.

^f R_{free} is calculated similarly to R_{factor} , except it uses 5% of the reflection data partitioned from the refinement process.

^g –, DNA and other solvent atoms are not present in the empty capsid structure.

and processing statistics for the empty capsids are summarized in Table 1, along with the data previously reported for the virions (40).

Structure determination of the H-1PV empty capsids. The structure determination of the H-1PV virions by the molecular replacement method is reported elsewhere (40). For the empty capsid structure determination, the orientation and position of capsids in the $P2_1$ unit cell were determined by molecular replacement procedures utilizing rotation and translation function searches conducted with a 60-mer C α model of the MVM VP2 crystal structure (Protein Data Bank [PDB] accession number 1z14 [31]) using the AMoRe program (43). The cross-rotation angles (Eulerian angles: $\alpha = 22.40^\circ$, $\beta = 70.89^\circ$, and $\gamma = 29.45^\circ$) and position (fractional coordinates of 0.2510, 0.0000, and 0.2497) for the capsids were similar to those observed for the virions (40). The correlation coefficient (CC) and R_{factor} for this molecular replacement solution were 0.4 and 43.8%, respectively, where $\text{CC} = (\sum |F_o| \times |F_c|) / [(\sum |F_o|^2) \times (\sum |F_c|^2)]^{1/2}$ and $R_{\text{factor}} = (\sum |F_o| - |F_c| / \sum |F_o|) \times 100$. F_o are the observed structure factors and F_c are the structure factors calculated from the model. The MVM C α capsid was rotated and translated into the unit cell according to the molecular replacement solution for subsequent steps in structure refinement.

H-1PV structure refinement. Procedures for the first cycle of refinement of the H-1PV virions are reported elsewhere (40). A similar proce-

ture was followed for determining the empty capsid structure. An MVM VP2 polyaniline model (from PDB accession no. 1z14 [31]) was superimposed onto the reference VP monomer in the oriented and positioned C α capsid model for subsequent refinement steps using the CNS program (44). Initial phases were calculated to the 3.2-Å diffraction resolution limit of the data (Table 1). These phases were improved using simulated annealing, energy minimization, conventional position, and individual temperature factor refinement (atomic displacement, measured in Å²; termed B_{factor}) while applying strict 60-fold noncrystallographic symmetry (NCS). This procedure was followed by real-space electron density averaging using a VP2 molecular mask, also constrained by 60-fold NCS. For monitoring of the refinement process, 5% of the total data set was partitioned for an R_{free} calculation (the same as R_{factor} but calculated with a 5% randomly selected fraction of the total recorded reflections not included in the refinement) (45). Following the first cycle of refinement, electron density interpretable as residues 38 to 593 (last C-terminal residue according to VP2 numbering) of the H-1PV VP2 were built into the averaged sigma-weighted $2F_o - F_c$ electron density maps for the virion and empty capsid structures. This was done by interactive substitution, insertion, and deletion of amino acids relative to the initial MVM VP2 polyaniline model using the COOT program (46). Low sigma threshold ($<0.5\sigma$) density observed under the icosahedral 5-fold axis, N terminal to residue 38, was not modeled, because the chain direction could not be unambiguously determined. New phases were calculated and improved by several alternating cycles of refinement, real-space electron density averaging, and model rebuilding. To improve the quality of the map, density modification was carried out using the Density Modification subroutine in the CNS program, which performed solvent flattening and NCS averaging (44).

In addition to VP2 residues 38 to 593 built into the averaged capsid density maps, ordered density in the interior of the virion structure could be modeled as a sequential chain of 9-nt ssDNA plus a single unconnected deoxycytidine nucleotide (dC). Backbone sugar and phosphate groups could also be built into the density at the 5' end of the 9-nt chain, but the position and type of base could not be unambiguously assigned. The model building was guided by the ssDNA structures reported for MVM and CPV (PDB accession numbers 1z1c, 4dpv, and 1p5w) (30, 31, 36). This density was located in a pocket created by the reference VP monomer and an icosahedral 2-fold related VP monomer. The phosphate backbone of the ordered nucleotide chain is coordinated by two metal ions. These metal ions ($F_o - F_c$ density at a sigma threshold of $>4\sigma$) were initially modeled as water molecules but refined with very low B_{factor} values and were assigned as Mg²⁺ following a B_{factor} test for Na⁺, Ca²⁺, or Mg²⁺. The CONTACT subroutine in the program CNS and the LIGPLOT visualization program were used to analyze the VP-ssDNA interaction within the nucleotide binding pocket (44, 47). Significantly, this density was not observed in the empty capsid structure, and coordinates for DNA were not included in the MVM VP2 phasing model. Finally, in the virion structure, 131 solvent molecules (within hydrogen-bonding distances), 4 molecules of ethylene glycol (EDO), two Na⁺ ions, and one Cl⁻ ion were built into remaining unassigned averaged positive $F_o - F_c$ density (at sigma thresholds of $\geq 3.0\sigma$) with acceptable B_{factor} values. Several cycles of refinement and averaging procedures interspersed by visual inspection of the $2F_o - F_c$ and $F_o - F_c$ electron density maps and model building in COOT (46) were performed until there was no further improvement in the agreement between the F_o and F_c structure factors as monitored by an R_{factor} .

The quality of the refined virion and empty capsid structures was analyzed using the COOT and MOLPROBITY programs (46, 48). The root mean square deviations (RMSD) from ideal bond lengths and angles were obtained from the CNS program (44), and the average B_{factor} values for the VP2 models, solvent molecules, nucleotides, and ions were calculated using the MOLEMAN program (49). The figures were generated using the PYMOL program (50), UCSF-Chimera program (51), and LIGPLOT (47).

Structural comparison of H-1PV to other parvoviruses. The VP2 coordinates of MVM, CPV, FPV, and PPV (PDB accession numbers 1z14, 2cas, 1c8f, and 1k3v, respectively) (31–33, 35) were aligned with the refined H-1PV empty capsid structure using the secondary structure matching (SSM) program available in PDBfold (52). This program superposes C α positions and gives the overall RMSD for the C α positions aligned and the atomic distances between aligned C α positions. This was used to identify variable regions that contain two or more residues with C α atom distances of ≥ 2.0 Å between the superposed structures, as previously defined (31). The sequence and conformation of the ssDNA nucleotides observed in the H-1PV virions was compared to that of ssDNA nucleotides observed in a similar pocket in virion structures of CPV and MVM (PDB accession numbers 1z1c, 4dpv, and 1p5w) (30, 31, 36). The VP residues forming this conserved ssDNA binding pocket for these parvoviruses then were compared with respect to structural changes induced upon genome packaging.

Protein structure accession numbers. The refined VP2 coordinates for the H-1PV virion and empty capsid structures and the data set structure factors have been deposited in the RCSB PDB under accession numbers 4g0r and 4gbt, respectively.

GenBank accession numbers. H-1PV VP2 and VP1 amino acid sequences were deposited in GenBank under AFR44451.1 and AFR44450.1, respectively. The VP2 amino acid sequences for MVM, CPV, FPV, and PPV are NP_041248.1, NP_955539.1, AAC37929.1, and NP_757372.1, respectively.

RESULTS AND DISCUSSION

The structure of H-1PV VP2 reveals conserved and variable regions compared to other parvoviruses. The structure of the H-1PV virions and empty capsids were determined to 2.7- and 3.2-Å resolution, respectively. For both structures, residues 38 to 593 (H-1PV VP2 numbering) of the common VP1/VP2/VP3 (in virions) or VP1/VP2 (in empty capsids) (hereinafter referred to as VP2) were ordered in the icosahedrally averaged electron density maps (Fig. 1A and B). This lack of N-terminal ordering has also been reported for all parvovirus structures determined to date (except B19V [37]). Presumably, the low copy numbers of VP1 and VP2 in the virions/capsids or different conformations adopted by the VP1/2/3 N termini, which is incompatible with the 60-fold icosahedral averaging, and/or intrinsic disorder, are responsible for the lack of the N-terminal ordering (38). The refinement parameters for the virion and empty capsid VP2 structures are given in Table 1. These are within the range reported for other virus structures determined at a comparable resolution, as calculated by the Polygon subroutine (53) in the program PHENIX (54). The similarity of R_{factor} and R_{free} for virus structures results from the noncrystallographic icosahedral symmetry of the capsid that limits the ability to select random unique reflections for the R_{free} calculation.

The H-1PV VP2 structure of the virion and empty capsid have the common conserved parvovirus capsid VP topology. It contains the core eight-stranded antiparallel jelly roll motif, consisting of two β -sheets, BIDG and CHEF, common in most virus structures, with inserted loops between the strands, and an α helix (αA ; residues 128 to 138; VP2 numbering) located close to the icosahedral 2-fold axis (Fig. 1B). Small regions of additional antiparallel β -strands are observed in the loops between the strands as previously described for other parvoviruses (reviewed in references 38 and 55). The β -barrel motif represents $\sim 23\%$ of the VP2 sequence and forms the contiguous capsid shell. The loops clustered as a consequence of the icosahedral symmetry-relating VP2 monomers form the capsid surface topology (Fig. 1C and D). The

FIG 1 Structure of H-1PV. (A) Sections of the $2F_o - F_c$ electron density map, contoured at a threshold of 1.2σ (gray mesh) and showing residues involved in *cis*-peptide bonds. Coordinates are shown as a stick model: C, orange; O, red; N, blue. (B) Ribbon diagram of the VP2 monomer within a transparent density surface. The conserved β -barrel core motif (β BIDG- β CHEF; orange), α A helix (blue), N and C termini, DE and HI loops, and the interior and exterior sides of VP2 are labeled. (C) A 9-mer of VP2 monomers, showing the icosahedral reference (Ref) and 5-fold (5f1-5f5), 3-fold (3f1-3f2; 3f3), and 2-fold (2f) symmetry relationships. The C α atoms of the amino acid residues involved in *cis*-peptide bonds are depicted as spheres: P470 (green), P424 (blue), P428 (red), and H348 (cyan). (D) Radially color-coded surface representation of the H-1PV capsid (the order from capsid center to surface is blue-green-yellow-red). In panels B and C, the positions of icosahedral 2-, 3-, and 5-fold symmetry axes are depicted as a filled oval, triangle, and pentagon, respectively.

floor of the 2-fold axis is the thinnest region of the capsid, one polypeptide thick, and it contains the smallest number of inter-monomer interactions (Fig. 1B and C). Two of the small stretches of antiparallel strand structure, between β D and β E, form β -ribbons (DE loop) which icosahedrally cluster to form a cylindrical channel at the 5-fold axis (Fig. 1B and D). There are two depressions, one at and surrounding the 2-fold axis, and the other surrounding the 5-fold cylinder (Fig. 1D). The floor of the depression around the cylinder is lined by the HI loop (between strands β H and β I) that extends over adjacent 5-fold symmetry-related VP2s (Fig. 1C and D). Six large surface loops, two from each 3-fold symmetry-related monomer, interdigitate to form the mound-like protrusion at the icosahedral 3-fold axis (Fig. 1D). These loops are located between β E and β F and between β G and β H (Fig. 1B). The shoulder/side of the protrusion is formed by loops between β B and β C and between β G and β H.

In addition to N-terminal residues 1 to 37, two and four amino acid residues were disordered at the radial extremity of the DE loop in the virion and empty capsid structures, respectively. Residues assigned in this capsid region for other parvovirus structures, including those of CPV and MVM, also have high thermal motion (31, 32, 35). This is likely due to flexibility of the 5-fold cylinder, which would be required for its reported function as a portal for externalization of VP1u and the N terminus of VP2 during viral trafficking through the endocytic pathway and for genome encapsidation (56–59).

Superposition of the C α positions of H-1PV VP2 with the analogous VP2 structures of MVM, CPV, FPV, and PPV shows conservation of the β -barrel core and the α A helix and homologous

surface loop topologies (Fig. 2A). The VP2 C α positions superposed with H-1PV, with overall RMSDs of 0.59 to 1.00 Å, secondary structure homology of 69 to 90%, and C α alignment of $\sim$ 96 to $\sim$ 99%, which is higher than the VP2 sequence identities of 50 to 66% (Table 2). However, while overall topologies of these parvovirus VP2s are conserved, differences were observed at the apexes of the loops located between the secondary-structure elements (Fig. 2A). Kontou et al. (31) reported eight surface loop regions that showed structural variability (VR1 to VR8) when the crystal structure of MVM was compared to those of CPV, FPV, and PPV (Fig. 2A). Differences between H-1PV and MVM, CPV, FPV, and PPV are also observed in VR1 (152 to 176), VR2 (231 to 253), VR3 (297 to 311), VR4a (314 to 340), VR4b (421 to 433), VR5 (361 to 380), VR6 (388 to 398), VR7 (508 to 525), and VR8 (553 to 572) (H-1PV VP2 numbering) (Fig. 2A). However, in addition, H-1PV differs from the other viruses in a surface loop consisting of residues 86 to 106 (labeled VR0 in Fig. 2A) that includes a four-amino-acid insertion in H-1PV. This VR is located on the 3-fold protrusion between VR2, VR3, VR4a, and VR4b (Fig. 2B). Clustering of the VRs on the assembled three-dimensional (3D) capsid contributes to local capsid surface variations between the viruses that are mostly concentrated at or around the icosahedral symmetry axes (Fig. 2B). Consistently, several VRs have been mapped as conformational antigenic sites on the MVM (VR2 and VR4b) and CPV capsids (VR0, VR2, VR3, and VR4b) (60–62). Since structure dictates function, it is likely that the structural elements conserved in the parvovirus genus have evolved to facilitate efficient capsid assembly, such as the core β -strands and α A, and to enable host

FIG 2 Comparison of VP2 monomer structures of *Parvovirus* genus members. (A) Superposition of coil representations of VP2 of H-1PV (orange), MVM (blue), PPV (pink), FPV (yellow), and CPV (green). The variable regions (VRs) are labeled. The positions of icosahedral 2-, 3-, and 5-fold symmetry axes are depicted as in Fig. 1B. (B) Surface representation of H-1PV capsid showing regions assigned as VR0 (purple), VR1 (blue), VR2 (yellow), VR3 (forest green), VR4a (red), VR4b (green), VR5 (pink), VR6 (cyan), VR7 (orange), and VR8 (brown) highlighted.

cell-specific interactions, such as those involving the VRs, as required for successful infection.

Variable regions control host interactions for the members of the genus *Parvovirus*. Structural VRs also exist between highly (~98% identity) homologous parvovirus strains, such as the MVM prototype strain (MVMp) and MVM immunosuppressive

strain (MVMi), and CPV and FPV (reviewed in references 31 and 55). In MVM, VR2, VR5, VR6, and VR8, located at or close to the 2-fold axis, configure its sialic acid (SIA) receptor binding site and are implicated in regulating tissue tropism and pathogenicity (63). In CPV, VR5 and VR6 are involved in SIA binding (39, 64). Mutation of H-1PV VP2 residues H374 and I368, equivalent to MVMp residues K368 and I362, involved in its SIA binding interaction, resulted in a drastic (~90 and 50% for the H374R and I368S mutants, respectively) reduction in H-1PV SIA-mediated cell binding (63, 65). One of the ethylene glycol molecules bound to H-1PV, EDO2 (see below), is located close to this SIA site at the 2-fold depression and interacts with H-1PV residues Y402, T403, W404, and D405, equivalent to MVM residues Y396, T397, W398, and D399, respectively. Residues D399, Y396, and W398 constitute the MVM SIA binding pocket, and D399 is a known MVM tropism determinant (29, 63, 66). These observations suggest that common residues regulate the MVM and H-1PV interaction with SIA, an important determinant of infectivity and tissue tropism.

A role in species host range adaptation has also been described for the VRs of members of the *Parvovirus* genus. An MVMp host range switch mutant, MVMp-F1, that adapted in tissue culture to replicate in rat fibroblasts, which are normally nonpermissive to MVM, contains mutations at VP2 amino acid residues A334T, E384A, N554D, and I578L (67). The structurally equivalent residues in H-1PV, A340, E390, V560, and I584 are located in VR4a (A340), VR6 (E390), and VR8 (V560), which surround the 2-fold depression (Fig. 2B). While the changes in this MVMp variant, which is now able to productively infect rat cells, as does H-1PV (67), are not toward the H-1PV VP2 amino acid sequence, this study again points to the involvement of the structural variation in the 2-fold region of the capsid in host range determination and cell surface glycan receptor attachment, as suggested by previous cell binding and mutagenesis data (65).

Another example of VR involvement in tropism determination is provided by a recent comparative study of rodent parvoviruses H-1PV, LuIII, and MVM, which reported that variation in their tumor cell tropism is VP2 associated (68, 69). The oncolytic activity of LuIII was mapped to the capsid VP2 gene using viral chimeras generated between LuIII and MVM in which the VP2 was swapped between the viruses. While the structure of LuIII is unknown, the observed VP2 structural variations between MVM and H-1PV, which are also predicted to exist between these viruses and LuIII, likely dictate their differences in tumor tropism. However, the exact mechanisms underlying tumor cell recognition and oncosuppression by these viruses are yet to be fully understood.

Ethylene glycol ordered in the H-1PV virion structure may play a stabilizing role in intermonomer interactions. Four molecules of ethylene glycol, likely from the PEG in the crystallization

TABLE 2 RMSD in Cα positions for H-1PV VP2 compared to other parvoviruses

Virus	% Identity ^a	Maximum RMSD in Cα position between H-1PV and the other parvoviruses in VR regions (Å)										
		Overall	VR0	VR1	VR2	VR3	VR4a	VR4b	VR5	VR6	VR7	VR8
MVMp	66 (90) (98.5)	0.59	1.6–5.6	2.5–3.4	1.0–3.2	1.0–2.3	1.0–2.9	1.0–1.4	1.0–1.2	1.0–1.5	1.0–2.0	1.1–3.4
CPV	50 (83) (96.7)	0.94	1.0–5.9	1.1–4.3	1.0–3.6	1.0–5.1	1.0–2.5	1.0–2.0	1.0–7.3	1.1–2.5	1.3–2.6	1.1–5.4
FPV	50 (72) (97.4)	0.87	1.0–5.6	1.1–3.7	1.1–4.3	1.1–4.9	1.1–2.8	1.4–2.1	1.1–6.3	1.1–2.6	1.1–2.7	1.0–5.4
PPV	51 (69) (96.4)	1.00	1.1–6.6	1.6–4.4	1.1–4.9	1.0–5.4	1.0–3.6	1.0–2.0	1.0–1.8	1.0–5.0	1.2–4.0	1.1–5.7

^a Values are for VP2 sequence identity. Values in the first set of parentheses are for VP2 secondary structural homology. Values in the second set of parentheses are for percentages of Cα positions aligned in the ordered VP2 structure.

solution, were built into the H-1PV virion structure. The first, EDO1, is located at the base of the 3-fold protrusion and interacts with residues R299, I300, and T301 of the reference VP2 and residues W111 and P214 of the 3-fold related VP2. The second, EDO2, is located at the 2-fold depression in the vicinity of the SIA binding region and interacts with residues Y402, T403, W404, and D405 of the reference VP2. The third, EDO3, is located close to the base of the 3-fold protrusion and interacts with residues R299, T314, D318, R319, and F320 of the reference VP2 and a Na^+ ion. The fourth, EDO4, is located under the HI loop in the depression surrounding the 5-fold cylinder and interacts with residues Y205, K208, K386, and Q387 of the reference VP2 and P517 of the 5-fold symmetry-related VP2. The localization of these molecules between symmetry-related VPs suggests a role in capsid stabilization, perhaps in a manner analogous to that of previous predictions for protein secondary structures using molecular dynamic simulations (70).

***cis*-peptide bonds observed in the H-1PV structures are a common feature in *Parvovirus* genus member capsids, suggesting a role in capsid stability and/or assembly.** Four *cis*-peptide bonds (3 proline bonds, i.e., A423-P424, I427-P428, and Y469-P470, and 1 nonproline *cis*-peptide bond, H348-D349) were observed in the H-1PV virion and empty capsid structures (Fig. 1A and C). These *cis*-peptide bonds were identified based on the backbone geometry and fit into the averaged electron density map (Fig. 1A). Residue P470 is located close to the icosahedral 2-fold axis of symmetry just above the conserved α A helix, and the aromatic side chain of Y469 is involved in π - π stacking interactions with residue W131 in the α A helix. The other two Pro *cis*-peptide bonds (A423-P424 and I427-P428) are located on the same loop at the shoulder of the 3-fold protrusions and interact with other loops from the reference and 3-fold symmetry-related VP2. A423 interacts with D349 from a 3-fold related VP2 that is also in a *cis* configuration, while P424 interacts with residue N327 in the same VP2 monomer. I427 interacts with Y330 of the reference VP2 as well as A102 and Y103 of the 3-fold related monomer, and P428 interacts with N100 of a 3-fold related monomer. The non-Pro *cis*-peptide bond, H348-D349, is located on the top of the 3-fold protrusion.

Three (H348-D349, I427-P428, and Y469-P470) of the four *cis*-peptide bonds observed in H-1PV are conserved in at least one of the five parvovirus structures compared. The H348-D349 *cis*-peptide bond is maintained in CPV between Y342 and Y343, and residue H348 is conserved in MVM as H342. The I427-P428 *cis*-peptide bond is conserved in CPV and FPV between L422 and P423, while sequence similarity is maintained in MVM (V421-P422) and PPV (L418-E419). For the Y469-P470 bond, the π - π stacking interaction between equivalent residues at Y469 and W131 is also conserved in CPV, FPV, and PPV (32, 33, 35, 36). Finally, while the A423-P424 *cis*-peptide bond is not conserved, the sequence is conserved in MVM (A417-P418) and PPV (A414-P415) but not in CPV (I418-N419) or FPV (I418-N419). Another *cis*-peptide bond occurs in the PPV VP2 structure at N219-P220, but this loop is not present in H-1PV, MVM, CPV, and FPV.

An analysis of the published protein structures showed that only 0.03% of all X-Xnp and 5.2% of all X-Pro peptide bonds occur in the *cis* conformation (where X is any residue and Xnp is any non-Pro residue) (71). A Pro-Pro bond has the highest frequency to be in the *cis* form, followed by Tyr-Pro. The *cis*-peptide bonds, especially non-Pro *cis*-peptide bonds, are rare due to steric

strain, but, when present, they are usually located near functional sites and have been suggested to be important determinants of a protein's function and to serve as an energy reservoir that drives conformational changes (72). As an example, the A31-F32 *cis*-peptide bond in the human immunodeficiency virus 1 capsid protein, at the binding site for the antiviral assembly inhibitor drug CAP-1, was suggested to facilitate capsid assembly (73). Also, a *cis-trans* isomerization of P78 in the coat protein of bacteriophage MS2 is proposed to play a role in capsid assembly (74). For other parvoviruses, the loops that are involved in the interactions at the 3-fold axis of symmetry are very closely interdigitated, and the presence of *cis*-peptide bonds, as observed here, could impart flexibility to the loop that is locked after assembly. As reported by Rioloobos et al. and others (75–77), interactions between VP2 monomers that interdigitate to assemble the 3-fold protrusions are critical for capsid assembly and stability. Thus, it is possible that these conserved *cis*-peptides play a major role in folding/unfolding of the loops to allow efficient capsid assembly.

Comparison of the H-1PV virion and empty capsid structures suggests that genome packaging alters the VP structure. A chain of 9 nt and an unconnected dC in the H-1PV virion structure (per VP2) were ordered in an interior pocket formed by the β -sheets of three VP2: the reference, a 3-fold related, and a 5-fold related VP2 (Fig. 3A and B). The dC density was connected to low sigma threshold density extending toward the interior of the capsid that was too disordered for assignment of additional nucleotides. The chain direction of the nucleotides was evident from high-sigma-threshold ($>5\sigma$) positive-difference density for the phosphate groups in the $F_o - F_c$ map. The resolution and quality of the icosahedrally averaged electron density map enabled distinction between purine (R) and pyrimidine (Y) bases, and for some nucleotides, the assignment of A or G and C or T was unambiguous (Fig. 3A). The DNA sequence 5'-NTGACTTCAA-3' (bold-face indicates unambiguous bases, and N is any base) was built as a chain of 9 nt plus an unassigned base, represented by N (Fig. 3A). With an occupancy of one, the average B_{factor} values for terminal nucleotides dN1, dA9, and dA10 (base type followed by nucleotide number) in this chain were high at 103, 105, and 125 $\text{\AA}^2$, respectively, while the average B_{factor} value for other nucleotides was $\sim 70 \text{ \AA}^2$. The B_{factor} value for the dC nucleotide is 122 $\text{\AA}^2$, while that of its cytosine base atoms is $\sim 73 \text{ \AA}^2$. Despite these high values, the assignments of the nucleotides were supported by high-sigma-threshold $F_o - F_c$ density (≥ 3.0) and the fit of the modeled bases (Fig. 3A). The high B_{factor} values also could be due to lower occupancy of the nucleotides compared to the VP amino acids.

The bases in the chain of 9 nt point radially outward to interact with the VP amino acids, while the looped deoxyribose-phosphate backbone has its phosphates on the inside of the loop chelated by two Mg^{2+} ions (Fig. 3A and 4). One Mg^{2+} ion is coordinated octahedrally by four phosphates, contributed by nucleotides dA4, dT6, dT7, and dA9 and VP residue N187. The second Mg^{2+} ion is chelated by the phosphates of nucleotides dN1, dG3, dT7, and dA9 and VP residue S186. The ssDNA is also stabilized via base-base stacking interactions, with two distinct stacks observed: dT6, dT7, dC8, and dA10 as well as dG3 and dA9 (Fig. 3A). The 5' and 3' ends of the looped DNA strand extend toward the 5-fold axis (Fig. 3B and C). Location of the 5' end of the DNA loop proximal to the 5-fold axis is consistent with reports that DNA packaging occurs via this channel in the 3' to 5' direction, and that the 5' end is located outside the capsid covalently linked to the NS1 protein

FIG 3 Ordered ssDNA in the H-1PV virion. (A) The assigned ssDNA nucleotides are shown inside the $2F_o - F_c$ electron density map (gray mesh) contoured at a threshold of 1.0σ . The 5' and 3' ends, Mg^{2+} ions (green spheres), and the nucleotides are labeled. Unambiguously assigned nucleotides are underlined. (B) The conserved nucleotide binding pocket in the capsid interior showing the ssDNA in H-1PV (cyan), MVM (blue), and CPV (green). The symmetry-related monomers (Ref, 3f, and 5f) interacting with the DNA are labeled. (C and D) A cross-section of the H-1PV virion (orange) with ordered DNA (nucleotide chain in cyan spheres; a single dC is shown as a stick model), not sectioned, viewed down the 2-fold axis (C) and rotated 90° for a sideways view (D). The ordered ssDNA forms a dodecahedral cage. The 5' (blue) and 3' ends (red) of the ssDNA strand are labeled. In panels B and C, the positions of the icosahedral 2-, 3-, and 5-fold symmetry axes are depicted as in Fig. 1B.

in newly packaged virions (57, 58, 78, 79). The dC nucleotide is located adjacent to the 2-fold axis (Fig. 3B and C).

The virion VP-DNA interactions for the 9-nt chain include nonspecific (with backbone and deoxyribose sugar) and specific (with nucleotide base atoms) van der Waals and polar interactions (Fig. 4 and 5A and Table 3). The binding site contains several residues that are within acceptable (2.6 to 4.0 Å) hydrogen-bonding and hydrophobic interaction distances, with 23 amino acid residues involved in base-specific interactions. The VP-DNA interactions are also stabilized through π - π stacking interactions between bases and amino acid side chains, for example, between the base of dG3 and residue Y275 and between the base of dC5 and residue H590 (Fig. 5A and Table 3). The nucleotide at position 5, dC5, flips out of base stacking to interact with residues located in a capsid pocket, and dT2 is oriented to interact with several residues in a different pocket (Fig. 5A and Table 3). The single dC nucleotide is involved in hydrogen-bonding interactions with G56 and K540, van der Waals contact with P281, and π - π stacking interactions between W59 and the cytosine base (not shown). Residues (Table 3) involved in these interactions, 51 to 56, 59, 146 to 148, 184, 272 to 281, 496 to 501, and 540, are located within β

strands βA , βB , βD , βE , βG , βH , and βI , respectively. Residue 57 is located at the turn between βA and βB ; residues 186 to 189 are present in a turn between βE and βF , while residues 285, 480, 545, 547, and 590 are present in loop regions.

The VP2 of the H-1PV virion and empty capsids superposed with an overall RMSD of 0.30 Å for all 552 (aa 38 to 593) residues. These structures were identical, except for side chain conformation differences observed at the DNA binding pocket. These occurred at residues K53, F54, L55, D57, Q148, D185, S186, P281, N497, K540, and H590 (Fig. 5A). Residues K53, F54, D57, Q148, D185, S186, N497, and H590 interact with the 9-nt chain and have side chains oriented closer to the DNA coordinates (ordered in the virion) in the empty capsid structure (Fig. 5A). In the virion, H590 adopts a dual conformation, with its imidazole ring involved in π - π stacking with the base of the dC5 nucleotide in one conformer (occupancy of 0.3 for this conformer), as mentioned above. In the empty capsid structure, H590 exists in only one conformer oriented differently from the two conformations adopted in the H-1PV virion structure (Fig. 5A). Residues L55, P281, and K540 have side chain orientations closer (L55) or further away (P281 and K540) from the single dC nucleotide in the empty cap-

FIG 4 VP-nucleotide interactions in H-1PV. VP2 amino acids within the hydrogen-bonding interaction distance of 2.6 to 3.6 Å to the ordered nucleotides are depicted. This image was generated by Ligplot. C, N, O, P, and Mg^{2+} are depicted as black, blue, red, cyan, and green spheres, respectively. The interaction key is provided.

sid compared to the virion structure (not shown). These observations indicate that a conformational rearrangement of interior capsid residues follows genome packaging.

The conserved parvovirus ssDNA binding pocket. The ssDNA ordered in the H-1PV virion is located in a DNA binding pocket previously reported for CPV and MVMi, for which virion structures are also available (29–31, 36) (Fig. 5B, C, and D). The 9-nt chain overlaps with a chain of nucleotides in MVMi (chain B; nucleotides 3 to 14; 5'-ATCCTCTATCAC-3') and CPV (chain N; nucleotides 1 to 11; 5'-NTACCTCTTGC-3') (PDB accession numbers 1z1c, 4dpv, and 1p5w) (30, 31, 36). This accounts for ~12% of the packaged genome for these viruses. The single dC nucleotide in H-1PV overlaps with one of the nucleotides (dA21) in a second oligonucleotide chain (chain C; nucleotides 15 to 23; 5'-ACACCAAAA-3') ordered in the MVMi structure (PDB accession number 1z1c) (31). Densities for the chain C nucleotides observed in MVMi were less defined in H-1PV and CPV and, thus, were not interpreted.

Remarkably, the chain of nucleotides ordered in the parvovirus genus ssDNA binding pocket adopts a similar conformation, including the flipping out of nucleotides located in positions equivalent to dT2 and dC5 of H-1PV, and they are stabilized by two divalent cations which are coordinated by the phosphates of nucleotides as well as VP residues (Fig. 5A to D). A similar coordination of Mg^{2+} ion with phosphate groups and a protein residue has been reported for other ssDNA binding proteins, for example, the I-PpoI endonuclease from *Physarum polycephalum* (80, 81).

The specific interactions between the H-1PV nucleotides and the VP involved capsid amino acid residues that are conserved in members of the genus *Parvovirus* (Table 4) and engage in similar VP-DNA interactions in CPV and MVMi (Fig. 5A, B, and C). Consistently, the nature of the ordered bases (R or Y) is conserved, particularly between H-1PV and CPV, with seven of the nine nucleotides being of the same type. For MVMi, four of the nine nucleotides structurally superposable to the H-1PV nucleotide chain are similarly assigned as R or Y. The significance of the insertion of nucleotides dT2 and dC5 into the VP2 for specific interactions is not yet known. However, the observation suggests that the ordered DNA is a potential recognition sequence or motif, and that the binding pocket residues are important for ssDNA recognition or stabilization.

The ordering of this ssDNA chain in parvoviruses is unusual, because these viruses package only one copy of their genome, and the structure determination procedure assumes icosahedral symmetry (60 equivalent positions). This suggests that at least 60 copies of a similar degenerate DNA sequence of R and Y is repeated in the ssDNA genome. Additionally, the observed overlap in sequence and structure for the nucleotides ordered in H-1PV, CPV, and MVMi, which are 67 to 83% identical at the genome level, suggests an important role for these VP-DNA interactions. Reguera et al. (82) reported that mutation of three of the ssDNA interacting residues in MVMi (Y270, D273, and D474, which are structurally equivalent to F266, D269, and D475 in CPV and Y275, N278, and D480 in H-1PV, respectively) to alanine drastically

FIG 5 Conformational variation in the parvovirus genus ssDNA binding pocket. (A, B, and C) The virion (pink) and empty capsid (yellow) VP2 structures of H-1PV, CPV, and MVMi, respectively, at their nucleotide binding pocket. The sugars and bases are colored according to atom type; N and O are in red and blue, respectively. The carbon atoms in the DNA and the phosphodiester backbone are colored in cyan (A), green (B), and blue (C), respectively. The amino acid residues and the nucleotides are labeled. The 5' and 3' ends and the Mg²⁺ ions (green spheres) are labeled. (D) Superposition of the ssDNA observed in H-1PV (cyan), CPV (green), and MVMi (blue). The nucleotide positions are labeled such that the first number represents the nucleotide position in H-1PV (similar to CPV) and the second represents that in MVMi.

reduced infectivity, with Y270A defective in capsid assembly, D273A unable to encapsidate genome or externalize VP2 N termini, and D474A showing reduced capsid stability. Mutations at other nucleotide interaction residues, D58, W60, N183, T267, K278, and K471, equivalent to N56, W58, N180, T263, R274, and K472 in CPV and D57, W59, N187, T272, K283, and K477 in H-1PV (Table 4), respectively, displayed reduced capsid stability (82). In another study, the MVM mutant F55A (F53 in CPV and F54 in H-1PV) yielded very low virus titers, which was attributed to a defect in either capsid assembly or stability (76). In addition, analysis of the mechanical properties of MVM virion and empty capsid suggests that the bound genome reinforces the stability of the particle, especially at the 2-fold symmetry axis (83). These reports thus support a role for the observed parvovirus VP-DNA interactions in capsid assembly, genome packaging, and increased stability of the packaged capsids. However, a role for genome packaging in capsid assembly is difficult to reconcile with reports that empty capsids are assembled prior to genome packaging for these parvoviruses (84, 85). A role for the observed ssDNA in capsid stability after assembly is supported by the dodecahedron DNA cage built by 60 copies of the 9-nt chain running along the seams between symmetry-related VP2 monomers (Fig. 3C and D).

Similar to members of the genus *Parvovirus*, icosahedrally ordered ssDNA has also been observed in the ssDNA-packaging bacteriophage Φ X174 (86). Significantly, similar to the interactions between the ordered parvovirus DNA and the capsid, the interactions between Φ X174 capsid residues and ssDNA involve residues in antiparallel β -strands, similar to that observed for other non-viral proteins which contain an oligonucleotide binding motif (OB fold) (87). This suggests that the viral ssDNA ordering is dictated by VP structure. Icosahedrally ordered RNA has been found in a number of viruses, including flock house virus, MS2, and satellite tobacco necrosis virus (STNV) (88–90). In MS2 and STNV, the capsid protein is involved in extensive contacts with ordered nucleotides (89, 90). For these viruses, structural, biochemical, and biophysical studies with RNA aptamers indicate that homologous (not identical) repeated RNA sequences, which occur in stem-loop structures, likely dictate the icosahedral ordering of observed nucleotides in the capsid structures (91–95). The ssDNA structure ordered in the parvoviruses also forms a loop, and ambiguity in the base assignment at some of the nucleotide positions could arise from the presence of homologous base types (i.e., a thymine or cytosine), rather than an identical base, at each of the 60 sites in the capsid. However, unlike RNA sequences, little information is available on the structure fold of ssDNA, particu-

TABLE 3 VP-nucleotide interactions in H-1PV virion

Nucleotide and VP atom	Nucleotide atom	Distance (Å)
dN1		
T272 (O) ^a	O4'	3.7
dT2		
T274 (O)	OP2	3.4
T272 (O)	C5'	3.2
L184 (CD2)	O4'	3.5
S186 (CB)	O3'/C1'	3.3/3.6
G501 (N)	C2	3.1
G501 (N/CA/C/O)	O2	2.7/2.9/3.6/3.6
S186 (CA)	O2	3.3
P500 (C)	N3	3.8
G501 (O/N)	N3	3.1/3.2
Q148 (NE2)	O4	2.8
L146 (CD2)	O4	3.6
L146 (O)	C7	3.3
S147 (CA)	C7	3.8
T272 (OG1/CB)	C7	3.0/3.6
G273 (O)	C7	3.2
Y275 (CB)	C7	3.6
dG3		
S186 (O)	OP1/C5'/C4'	3.6/3.4/3.5
N498 (ND2)	O4'/C4'	3.2/3.7
Y275 (CD2)	N1	3.6
Y275 (CE2)	N1/N2/C2	3.4/3.6/3.4
I276 (O)	N1/C2/N2/O6	2.8/3.5/3.2/3.8
I276 (N)	N1/C6/O6	3.6/3.5/2.7
Y275 (CZ/OH)	N2	3.6/3.3
N497 (ND2/CG/OD1)	N2	2.5/2.8/3.0
N498 (O)	N2	3.2
I276 (CA/CB)	O6	3.6/3.6
Y275 (C/CA)	O6	3.5/3.5
dA4		
N187 (OD1)	OP1	2.8
N187 (O)	C5'/C4'	3.4/3.5
N498 (OD1)	C4'/O4'	3.6/3.2
N498 (CG/CB)	O4'	3.0/3.0
K496 (O)	N1/C2	3.8/3.7
N497 (CA)	N1	3.6
dC5		
D480 (OD2)	OP2	3.3
H590 (NE2)	OP2	3.4
N187 (O/CB)	C5'/O3'	3.5/3.7
N188 (O)	C5'	3.5
I189 (CG1)	C4'	4.0
P545 (CG)	N1/C2	3.5/3.5
L547 (CD1)	O2	3.6
H590 (CE1)	N3/C4/C5/C6/N1/C2	3.5/3.3/3.3/3.5/3.7/3.7
H590 (ND1)	N3/C4/N4	3.2/3.1/3.3
H590 (CG)	C4/N4	3.5/3.3
H590 [CB(A/B)]	N4	3.2/3.5
H590 (NE2)	C5	3.4
T285 (CG2)	N4	3.5
dT6		
N187 (OD1/CG/CB)	OP1	3.0/3.2/3.6
I189 (CD1)	C5'	3.7
L547 (CD1)	C5'	3.8
L547 (CD2)	O4'	3.6
N187 (ND2)	O3'	3.3

TABLE 3 (Continued)

Nucleotide and VP atom	Nucleotide atom	Distance (Å)
Y52 (N/O)	C2	3.5/3.7
T51 (CA/CB/C)	O2	3.1/3.4/3.3
Y52 (O/N/CA)	O2	3.6/2.6/3.6
Y52 (O/C/CB)	N3	3.0/3.9/3.8
Y52 (O)	C4/O4	3.9/4.0
F54 (CE1)	O4	3.6
P545 (CB)	C5/C7	3.9/3.6
dT7		
N187 (ND2)	P/OP1/OP2	3.4/3.1/3.5
N187 (OD1)	OP2	3.5
T51 (CG2/CB)	O4'	3.5/3.6
Y52 (O)	C2/O2/N3/C4	3.4/3.2/3.0/4.0
Y52 (C)	O2/N3	3.5/4.0
K53 (CA/CB/N)	O2	3.4/3.6/3.6
dC8		
D57 (OD2)	N3/C4/N4	3.8/4.0/3.4/2.9
K53 (CD/CE)	N4	3.0/3.2
dA9		
N187 (OD1)	OP2	3.0
dA10		
D57 (OD1)	N6	3.3
dC		
P281 (CG)	OP1	3.6
W59 (CH2)	O5'/C5'	3.6/3.5
W59 (CZ2)	C5'	3.7
W59 (CE3)	C2'	3.7
K540 (NZ)	O3'/C2'	3.1/3.6
L55 (CD2)	O2	4.0
W59 (CD1)	N3/C4/C5	3.6/3.3/3.4
G56 (O)	N4	3.8
W59 (NE1)	C5/C6	3.4/3.5

^a VP atoms participating in DNA interaction.

larly those ordered in viruses. Efforts to use a nucleic acid folding algorithm, as employed by MFOLD (96), to predict the structure of the parvovirus member genomes produced inconclusive results due to the significant influence of Mg²⁺ in structure topology (not shown). It is still to be determined whether genome sequence, genome structure, VP structural elements, or VP sequence dictates the genome ordering in these viruses.

Summary. A comparative analysis of parvovirus structures, as reported here, provides the basis for functional annotation of the capsid with respect to the requirements for successful infection. It also provides information about conserved capsid regions that are required for efficient assembly. The structural differences observed between H-1PV and MVM capsids provide a basis for future molecular studies to decipher their mechanism of differential tumor tropism and toxicity. The structure also identifies capsid regions that can tolerate loop insertions/deletions and incorporation of targeting peptides without disrupting capsid assembly or genome packaging. This provides a framework for engineering (i) mosaic/chimeric vectors by the shuffling of capsid genes from related oncotropic autonomous parvoviruses or (ii) capsids with peptide insertions with improved cancer cell targeting. The ordering of ssDNA in a conserved parvovirus nucleotide binding site in

TABLE 4 Amino acid sequence at the nucleotide binding pocket in members of the genus *Parvovirus*

Amino acid residues		
H-1PV	MVM	CPV
Amino acid residues interacting with 9-nt chain		
T51	H52	E50
Y52	Y53	F51
K53	R54	K52
F54	F55	F53
D57	D58	N56
L146	L143	F141
S147	D144	E142
Q148	Q145	Q143
L184	V180	L177
S186	S182	S179
N187	N183	N180
N188	N184	N181
I189	I185	T182
T272	T267	T263
G273	G268	G264
T274	T269	T265
Y275	Y270	F266
I276	Y271	F267
T285	T280	T276
D480	D474	D475
K496	K490	Q491
N497	N491	N492
N498	N492	N493
P500	P494	P495
G501	G495	G496
P545	A539	A541
L547	T541	H543
H590	R584	R581
Amino acid residues interacting with dC		
L55	L56	L54
G56	G57	E55
W59	W60	W58
P281	P276	P272
K540	R534	K536

the H-1PV virion indicates a potential role in capsid stability, following genome packaging, for the contact residues that can inform the engineering of vectors with improved packaging capability.

ACKNOWLEDGMENTS

We thank the staff at the X29 beamline at NSLS, Brookhaven National Laboratory, and the F1 beamline at CHESS, Cornell University, for help during data collection. We especially thank Kathy Dedrick at CHESS for assistance in obtaining beam time.

The NSLS is supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under contract no. DE-AC02-98CH10886. CHESS is supported by the National Science Foundation and the National Institutes of Health/National Institute of General Medical Sciences under NSF award DMR-0936384, and the MacCHESS facility is funded through award GM-103485 from the National Institute of General Medical Sciences, National Institutes of Health.

This project was funded by NSF project MCB-0718948 (R.M. and M.A.-M.) and UF COM Research Funds to (M.A.-M.).

REFERENCES

- Berns K, Parrish CR. 2007. Parvoviridae, p 2437–2478. In Knipe DM, Howley PM, Griffin DE, Lamb RA, Martin MA, Roizman B, Straus SE (ed), Fields virology, 5th ed. Lippincott Williams & Wilkins, Philadelphia, PA.
- Toolan HW, Dalldore G, Barclay M, Chandra S, Moore AE. 1960. An unidentified, filtrable agent isolated from transplanted human tumors. Proc. Natl. Acad. Sci. U. S. A. 46:1256–1258.
- Toolan HW. 1968. The picodna viruses. H, RV, and AAV. Int. Rev. Exp. Pathol. 6:135–180.
- Blechacz B, Russell SJ. 2004. Parvovirus vectors: use and optimisation in cancer gene therapy. Expert Rev. Mol. Med. 6:1–24.
- Rommelaere J, Geletneky K, Angelova AL, Daefluer L, Dinsart C, Kiprianova I, Schlehofer JR, Raykov Z. 2010. Oncolytic parvoviruses as cancer therapeutics. Cytokine Growth Factor Rev. 21:185–195.
- Spalholz BA, Tattersall P. 1983. Interaction of minute virus of mice with differentiated cells: strain-dependent target cell specificity is mediated by intracellular factors. J. Virol. 46:937–943.
- Cotmore SF, Tattersall P. 1987. The autonomously replicating parvoviruses of vertebrates. Adv. Virus Res. 33:91–174.
- Angelova AL, Aprahamian M, Balboni G, Delecluse HJ, Feederle R, Kiprianova I, Grekova SP, Galabov AS, Witzens-Harig M, Ho AD, Rommelaere J, Raykov Z. 2009. Oncolytic rat parvovirus H-1PV, a candidate for the treatment of human lymphoma: *in vitro* and *in vivo* studies. Mol. Ther. 17:1164–1172.
- Angelova AL, Aprahamian M, Grekova SP, Hajri A, Leuchs B, Giese NA, Dinsart C, Herrmann A, Balboni G, Rommelaere J, Raykov Z. 2009. Improvement of gemcitabine-based therapy of pancreatic carcinoma by means of oncolytic parvovirus H-1PV. Clin. Cancer Res. 15:511–519.
- Faisst S, Guittard D, Benner A, Cesbron JY, Schlehofer JR, Rommelaere J, Dupressoir T. 1998. Dose-dependent regression of HeLa cell-derived tumours in SCID mice after parvovirus H-1 infection. Int. J. Cancer 75: 584–589.
- Herrero Y, Calle M, Cornelis JJ, Herold-Mende C, Rommelaere J, Schlehofer JR, Geletneky K. 2004. Parvovirus H-1 infection of human glioma cells leads to complete viral replication and efficient cell killing. Int. J. Cancer 109:76–84.
- Malerba M, Daefluer L, Rommelaere J, Iggo RD. 2003. Replicating parvoviruses that target colon cancer cells. J. Virol. 77:6683–6691.
- Van Pachterbeke C, Tuynder M, Brandenburger A, Leclercq G, Borras M, Rommelaere J. 1997. Varying sensitivity of human mammary carcinoma cells to the toxic effect of parvovirus H-1. Eur. J. Cancer 33:1648–1653.
- Enderlin M, Kleinmann EV, Struyf S, Buracchi C, Vecchi A, Kinscherf R, Kiessling F, Paschek S, Sozzani S, Rommelaere J, Cornelis JJ, Van Damme J, Dinsart C. 2009. TNF- α and the IFN- γ -inducible protein 10 (IP-10/CXCL-10) delivered by parvoviral vectors act in synergy to induce antitumor effects in mouse glioblastoma. Cancer Gene Ther. 16:149–160.
- Haag A, Menten P, Van Damme J, Dinsart C, Rommelaere J, Cornelis JJ. 2000. Highly efficient transduction and expression of cytokine genes in human tumor cells by means of autonomous parvovirus vectors; generation of antitumor responses in recipient mice. Hum. Gene Ther. 11:597–609.
- Moehler M, Sieben M, Roth S, Springsguth F, Leuchs B, Zeidler M, Dinsart C, Rommelaere J, Galle PR. 2011. Activation of the human immune system by chemotherapeutic or targeted agents combined with the oncolytic parvovirus H-1. BMC Cancer 11:464. doi:10.1186/1471-2407-11-464.
- Moehler M, Zeidler M, Schede J, Rommelaere J, Galle PR, Cornelis JJ, Heike M. 2003. Oncolytic parvovirus H1 induces release of heat-shock protein HSP72 in susceptible human tumor cells but may not affect primary immune cells. Cancer Gene Ther. 10:477–480.
- Olijslagers S, Dege AY, Dinsart C, Voorhoeve M, Rommelaere J, Noteborn MH, Cornelis JJ. 2001. Potentiation of a recombinant oncolytic parvovirus by expression of Apoptin. Cancer Gene Ther. 8:958–965.
- Wetzel K, Menten P, Opdenakker G, Van Damme J, Gröne HJ, Giese N, Vecchi A, Sozzani S, Cornelis JJ, Rommelaere J, Dinsart C. 2001. Transduction of human MCP-3 by a parvoviral vector induces leukocyte infiltration and reduces growth of human cervical carcinoma cell xenografts. J. Gene Med. 3:326–337.
- Toolan HW, Saunders EL, Southam CM, Moore AE, Levin AG. 1965. H-1 virus viremia in the human. Proc. Soc. Exp. Biol. Med. 119:711–715.
- Lacroix J, Leuchs B, Li J, Hristov G, Deubzer HE, Kulozik AE, Rommelaere J, Schlehofer JR, Witt O. 2010. Parvovirus H1 selectively induces

- cytotoxic effects on human neuroblastoma cells. *Int. J. Cancer* 127:1230–1239.
22. Moehler M, Blechacz B, Weiskopf N, Zeidler M, Stremmel W, Rommelaere J, Galle PR, Cornelis JJ. 2001. Effective infection, apoptotic cell killing and gene transfer of human hepatoma cells but not primary hepatocytes by parvovirus H1 and derived vectors. *Cancer Gene Ther.* 8:158–167.
 23. Geletnekky K, Kiprianova I, Ayache A, Koch R, Herrero Y, Calle M, Deleu L, Sommer C, Thomas N, Rommelaere J, Schlehofer JR. 2010. Regression of advanced rat and human gliomas by local or systemic treatment with oncolytic parvovirus H-1 in rat models. *Neurol. Oncol.* 12: 804–814.
 24. Kiprianova I, Thomas N, Ayache A, Fischer M, Leuchs B, Klein M, Rommelaere J, Schlehofer JR. 2011. Regression of glioma in rat models by intranasal application of parvovirus H-1. *Clin. Cancer Res.* 17:5333–5342.
 25. Geletnekky K, Huesing J, Rommelaere J, Schlehofer JR, Leuchs B, Dahm M, Krebs O, von Knebel Doeberitz M, Huber B, Hajda J. 2012. Phase I/IIa study of intratumoral/intracerebral or intravenous/intracerebral administration of parvovirus H-1 (ParvOryx) in patients with progressive primary or recurrent glioblastoma multiforme: ParvOryx01 protocol. *BMC Cancer* 12:99. doi:10.1186/1471-2407-12-99.
 26. Legrand C, Rommelaere J, Caillet-Fauquet P. 1993. MVM(p) NS-2 protein expression is required with NS-1 for maximal cytotoxicity in human transformed cells. *Virology* 195:149–155.
 27. Tattersall P, Cawte PJ, Shatkin AJ, Ward DC. 1976. Three structural polypeptides coded for by minute virus of mice, a parvovirus. *J. Virol.* 20:273–289.
 28. Agbandje M, McKenna R, Rossmann MG, Strassheim ML, Parrish CR. 1993. Structure determination of feline panleukopenia virus empty particles. *Proteins* 16:155–171.
 29. Agbandje-McKenna M, Llamas-Saiz AL, Wang F, Tattersall P, Rossmann MG. 1998. Functional implications of the structure of the murine parvovirus, minute virus of mice. *Structure* 6:1369–1381.
 30. Govindasamy L, Hueffer K, Parrish CR, Agbandje-McKenna M. 2003. Structures of host range-controlling regions of the capsids of canine and feline parvoviruses and mutants. *J. Virol.* 77:12211–12221.
 31. Kontou M, Govindasamy L, Nam HJ, Bryant N, Llamas-Saiz AL, Foces-Foces C, Hernando E, Rubio MP, McKenna R, Almendral JM, Agbandje-McKenna M. 2005. Structural determinants of tissue tropism and in vivo pathogenicity for the parvovirus minute virus of mice. *J. Virol.* 79:10931–10943.
 32. Simpson AA, Chandrasekar V, Hébert B, Sullivan GM, Rossmann MG, Parrish CR. 2000. Host range and variability of calcium binding by surface loops in the capsids of canine and feline parvoviruses. *J. Mol. Biol.* 300: 597–610.
 33. Simpson AA, Hébert B, Sullivan GM, Parrish CR, Zádori Z, Tijssen P, Rossmann MG. 2002. The structure of porcine parvovirus: comparison with related viruses. *J. Mol. Biol.* 315:1189–1198.
 34. Tsao J, Chapman MS, Agbandje M, Keller W, Smith K, Wu H, Luo M, Smith TJ, Rossmann MG, Compans RW, Parrish CR. 1991. The three-dimensional structure of canine parvovirus and its functional implications. *Science* 251:1456–1464.
 35. Wu H, Rossmann MG. 1993. The canine parvovirus empty capsid structure. *J. Mol. Biol.* 233:231–244.
 36. Xie Q, Chapman MS. 1996. Canine parvovirus capsid structure, analyzed at 2.9 Å resolution. *J. Mol. Biol.* 264:497–520.
 37. Kaufmann B, Chipman PR, Kostyuchenko VA, Modrow S, Rossmann MG. 2008. Visualization of the externalized VP2 N termini of infectious human parvovirus B19. *J. Virol.* 82:7306–7312.
 38. Chapman MS, Agbandje-McKenna M. 2006. Atomic structure of viral particles, p 107–123. *In* Kerr JR, Cotmore SF, Bloom ME, Linden RM, Parrish CR (ed), *Parvoviruses*. Hodder Arnold, London, United Kingdom.
 39. Agbandje-McKenna M, Chapman MS. 2006. Correlating structure with function in viral capsid, p 125–139. *In* Kerr JR, Cotmore SF, Bloom ME, Linden RM, Parrish CR (ed), *Parvoviruses*. Hodder Arnold, London, United Kingdom.
 40. Halder S, Nam HJ, Govindasamy L, Vogel M, Dinsart C, Salomé N, McKenna R, Agbandje-McKenna M. 2012. Production, purification, crystallization and structure determination of H-1 parvovirus. *Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun.* 68:1571–1576.
 41. Otwinowski Z, Minor W. 1997. Processing of X-ray diffraction data collected in oscillation mode. *Methods Enzymol.* 276:307–326.
 42. Matthews BW. 1968. Solvent content of protein crystals. *J. Mol. Biol.* 33:491–497.
 43. Navaza J. 2001. Implementation of molecular replacement in *AMoRe*. *Acta Crystallogr. D. Biol. Crystallogr.* 57:1367–1372.
 44. Brünger AT, Adams PD, Clore GM, DeLano WL, Gros P, Grosse-Kunstleve RW, Jiang JS, Kuszewski J, Nilges M, Pannu NS, Read RJ, Rice LM, Simonson T, Warren GL. 1998. Crystallography & NMR system: a new software suite for macromolecular structure determination. *Acta Crystallogr. D. Biol. Crystallogr.* 54:905–921.
 45. Brünger AT. 1992. Free *R* value: a novel statistical quantity for assessing the accuracy of crystal structures. *Nature* 355:472–475.
 46. Emsley P, Lohkamp B, Scott WG, Cowtan K. 2010. Features and development of *Coot*. *Acta Crystallogr. D. Biol. Crystallogr.* 66:486–501.
 47. Wallace AC, Laskowski RA, Thornton JM. 1995. LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. *Protein Eng.* 8:127–134.
 48. Chen VB, Arendall WB, III, Headd JJ, Keedy DA, Immormino RM, Kapral GJ, Murray LW, Richardson JS, Richardson DC. 2010. MolProbity: all-atom structure validation for macromolecular crystallography. *Acta Crystallogr. D. Biol. Crystallogr.* 66:12–21.
 49. Kleywegt GJ, Jones TA. 1997. Model building and refinement practice. *Methods Enzymol.* 277:208–230.
 50. DeLano WL. 2002. The PyMOL molecular graphics system. DeLano Scientific LLC, San Carlos, CA.
 51. Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE. 2004. UCSF Chimera—a visualization system for exploratory research and analysis. *J. Comput. Chem.* 25:1605–1612.
 52. Krissinel E, Henrick K. 2004. Secondary-structure matching (SSM), a new tool for fast protein structure alignment in three dimensions. *Acta Crystallogr. D. Biol. Crystallogr.* 60:2256–2268.
 53. Urzhumtseva L, Afonine PV, Adams PD, Urzhumtsev A. 2009. Crystallographic model quality at a glance. *Acta Crystallogr. D. Biol. Crystallogr.* 65:297–300.
 54. Adams PD, Afonine PV, Bunkóczi G, Chen VB, Davis IW, Echols N, Headd JJ, Hung LW, Kapral GJ, Grosse-Kunstleve RW, McCoy AJ, Moriarty NW, Oeffner R, Read RJ, Richardson DC, Richardson JS, Terwilliger TC, Zwart PH. 2010. PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr. D. Biol. Crystallogr.* 66:213–221.
 55. Halder S, Ng R, Agbandje-McKenna M. 2012. Parvoviruses: structure and infection. *Future Virol.* 7:253–278.
 56. Cotmore SF, Tattersall P. 2007. Parvoviral host range and cell entry mechanisms. *Adv. Virus Res.* 70:183–232.
 57. Farr GA, Cotmore SF, Tattersall P. 2006. VP2 cleavage and the leucine ring at the base of the fivefold cylinder control pH-dependent externalization of both the VP1 N terminus and the genome of minute virus of mice. *J. Virol.* 80:161–171.
 58. Farr GA, Tattersall P. 2004. A conserved leucine that constricts the pore through the capsid fivefold cylinder plays a central role in parvoviral infection. *Virology* 323:243–256.
 59. Farr GA, Zhang LG, Tattersall P. 2005. Parvoviral virions deploy a capsid-tethered lipolytic enzyme to breach the endosomal membrane during cell entry. *Proc. Natl. Acad. Sci. U. S. A.* 102:17148–17153.
 60. Hafenstein S, Bowman VD, Sun T, Nelson CD, Palermo LM, Chipman PR, Battisti AJ, Parrish CR, Rossmann MG. 2009. Structural comparison of different antibodies interacting with parvovirus capsids. *J. Virol.* 83: 5556–5566.
 61. Kaufmann B, López-Bueno A, Mateu MG, Chipman PR, Nelson CD, Parrish CR, Almendral JM, Rossmann MG. 2007. Minute virus of mice, a parvovirus, in complex with the Fab fragment of a neutralizing monoclonal antibody. *J. Virol.* 81:9851–9858.
 62. Wikoff WR, Wang G, Parrish CR, Cheng RH, Strassheim ML, Baker TS, Rossmann MG. 1994. The structure of a neutralized virus: canine parvovirus complexed with neutralizing antibody fragment. *Structure* 2:595–607.
 63. López-Bueno A, Rubio MP, Bryant N, McKenna R, Agbandje-McKenna M, Almendral JM. 2006. Host-selected amino acid changes at the sialic acid binding pocket of the parvovirus capsid modulate cell binding affinity and determine virulence. *J. Virol.* 80:1563–1573.
 64. Barbis DP, Chang SF, Parrish CR. 1992. Mutations adjacent to the dimple of the canine parvovirus capsid structure affect sialic acid binding. *Virology* 191:301–308.
 65. Allaume X, El-Andaloussi N, Leuchs B, Bonifati S, Kulkarni A, Marttila

- T, Kaufmann JK, Nettelbeck DM, Kleinschmidt J, Rommelaere J, Marchini A. 2012. Retargeting of rat parvovirus H-1PV to cancer cells through genetic engineering of the viral capsid. *J. Virol.* 86:3452–3465.
66. D'Abramo AM, Jr, Ali AA, Wang F, Cotmore SF, Tattersall P. 2005. Host range mutants of minute virus of mice with a single VP2 amino acid change require additional silent mutations that regulate NS2 accumulation. *Virology* 340:143–154.
 67. Etingov I, Itah R, Minberg M, Keren-Naus A, Nam HJ, Agbandje-McKenna M, Davis C. 2008. An extension of the minute virus of mice tissue tropism. *Virology* 379:245–255.
 68. Paglino J, Tattersall P. 2011. The parvoviral capsid controls an intracellular phase of infection essential for efficient killing of stepwise-transformed human fibroblasts. *Virology* 416:32–41.
 69. Paglino JC, Ozduman K, van den Pol AN. 2012. LuIII parvovirus selectively and efficiently targets, replicates in, and kills human glioma cells. *J. Virol.* 86:7280–7291.
 70. de Oliveira OV, de Moura AF, Freitas LCG. 2007. A mechanism for the stabilization of the secondary structure of a peptide by liquid ethylene glycol and its aqueous solutions. *J. Mol. Struct. Theochem.* 808:93–96.
 71. Weiss MS, Jabs A, Hilgenfeld R. 1998. Peptide bonds revisited. *Nat. Struct. Biol.* 5:676.
 72. Brandts JF, Halvorson HR, Brennan M. 1975. Consideration of the possibility that the slow step in protein denaturation reactions is due to cis-trans isomerism of proline residues. *Biochemistry* 14:4953–4963.
 73. Kelly BN, Kyere S, Kinde I, Tang C, Howard BR, Robinson H, Sundquist WI, Summers MF, Hill CP. 2007. Structure of the antiviral assembly inhibitor CAP-1 complex with the HIV-1 CA protein. *J. Mol. Biol.* 373:355–366.
 74. Golmohammadi R, Valegård K, Fridborg K, Liljas L. 1993. The refined structure of bacteriophage MS2 at 2.8 Å resolution. *J. Mol. Biol.* 234:620–639.
 75. Pérez R, Castellanos M, Rodríguez-Huete A, Mateu MG. 2011. Molecular determinants of self-association and rearrangement of a trimeric intermediate during the assembly of a parvovirus capsid. *J. Mol. Biol.* 413:32–40.
 76. Reguera J, Carreira A, Riobobos L, Almendral JM, Mateu MG. 2004. Role of interfacial amino acid residues in assembly, stability, and conformation of a spherical virus capsid. *Proc. Natl. Acad. Sci. U. S. A.* 101:2724–2729.
 77. Riobobos L, Reguera J, Mateu MG, Almendral JM. 2006. Nuclear transport of trimeric assembly intermediates exerts a morphogenetic control on the icosahedral parvovirus capsid. *J. Mol. Biol.* 357:1026–1038.
 78. Cotmore SF, Hafenstein S, Tattersall P. 2010. Depletion of virion-associated divalent cations induces parvovirus minute virus of mice to eject its genome in a 3'-to-5' direction from an otherwise intact viral particle. *J. Virol.* 84:1945–1956.
 79. Cotmore SF, Tattersall P. 2012. Mutations at the base of the icosahedral five-fold cylinders of minute virus of mice induce 3'-to-5' genome uncoating and critically impair entry functions. *J. Virol.* 86:69–80.
 80. Batra VK, Beard WA, Shock DD, Krahn JM, Pedersen LC, Wilson SH. 2006. Magnesium-induced assembly of a complete DNA polymerase catalytic complex. *Structure* 14:757–766.
 81. Galburt EA, Chevalier B, Tang W, Jurica MS, Flick KE, Monnat RJ, Jr, Stoddard BL. 1999. A novel endonuclease mechanism directly visualized for I-PpoI. *Nat. Struct. Biol.* 6:1096–1099.
 82. Reguera J, Grueso E, Carreira A, Sánchez-Martínez C, Almendral JM, Mateu MG. 2005. Functional relevance of amino acid residues involved in interactions with ordered nucleic acid in a spherical virus. *J. Biol. Chem.* 280:17969–17977.
 83. Carrasco C, Carreira A, Schaap IA, Serena PA, Gómez-Herrero J, Mateu MG, de Pablo PJ. 2006. DNA-mediated anisotropic mechanical reinforcement of a virus. *Proc. Natl. Acad. Sci. U. S. A.* 103:13706–13711.
 84. Richards R, Linser P, Armentrout RW. 1977. Kinetics of assembly of a parvovirus, minute virus of mice, in synchronized rat brain cells. *J. Virol.* 22:778–793.
 85. Yuan W, Parrish CR. 2001. Canine parvovirus capsid assembly and differences in mammalian and insect cells. *Virology* 279:546–557.
 86. McKenna R, Ilag LL, Rossmann MG. 1994. Analysis of the single-stranded DNA bacteriophage ϕ X174, refined at a resolution of 3.0 Å. *J. Mol. Biol.* 237:517–543.
 87. Theobald DL, Mitton-Fry RM, Wuttke DS. 2003. Nucleic acid recognition by OB-fold proteins. *Annu. Rev. Biophys. Biomol. Struct.* 32:115–133.
 88. Fisher AJ, Johnson JE. 1993. Ordered duplex RNA controls capsid architecture in an icosahedral animal virus. *Nature* 361:176–179.
 89. Lane SW, Dennis CA, Lane CL, Trinh CH, Rizkallah PJ, Stockley PG, Phillips SE. 2011. Construction and crystal structure of recombinant STNV capsids. *J. Mol. Biol.* 413:41–50.
 90. Valegård K, Murray JB, Stockley PG, Stonehouse NJ, Liljas L. 1994. Crystal structure of an RNA bacteriophage coat protein-operator complex. *Nature* 371:623–626.
 91. Borodavka A, Tuma R, Stockley PG. 2012. Evidence that viral RNAs have evolved for efficient, two-stage packaging. *Proc. Natl. Acad. Sci. U. S. A.* 109:15769–15774.
 92. Bunka DH, Lane SW, Lane CL, Dykeman EC, Ford RJ, Barker AM, Twarock R, Phillips SE, Stockley PG. 2011. Degenerate RNA packaging signals in the genome of satellite tobacco necrosis virus: implications for the assembly of a T=1 capsid. *J. Mol. Biol.* 413:51–65.
 93. Helgstrand C, Grahm E, Moss T, Stonehouse NJ, Tars K, Stockley PG, Liljas L. 2002. Investigating the structural basis of purine specificity in the structures of MS2 coat protein RNA translational operator hairpins. *Nucleic Acids Res.* 30:2678–2685.
 94. Lago H, Fonseca SA, Murray JB, Stonehouse NJ, Stockley PG. 1998. Dissecting the key recognition features of the MS2 bacteriophage translational repression complex. *Nucleic Acids Res.* 26:1337–1344.
 95. Stockley PG, Stonehouse NJ, Murray JB, Goodman ST, Talbot SJ, Adams CJ, Liljas L, Valegård K. 1995. Probing sequence-specific RNA recognition by the bacteriophage MS2 coat protein. *Nucleic Acids Res.* 23:2512–2518.
 96. Zuker M. 2003. Mfold web server for nucleic acid folding and hybridization prediction. *Nucleic Acids Res.* 31:3406–3415.