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The Capsid Architecture of Channel Catfish Virus, an Evolutionarily Distant Herpesvirus, with Herpes Simplex Virus

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Although herpesviruses have a wide host range and their genomes vary substantially in size, the nucleocapsid appears to be a conservative element of viral design. The capsid shell is icosahedrally symmetric ($T = 16$), and 125 nm in diameter and 15nm thick in the case of herpes simplex virus 1 (HSV-1). Channel catfish virus (CCV) has the gross morphology of a herpesvirus, although no relationship to other herpesviruses is evident from the sequences of its proteins. To examine CCV capsid architecture more closely, we have determined its structure by cryoelectron microscopy and three-dimensional image reconstruction. The CCV capsid is smaller than that of HSV-1, but its 12% smaller genome is packed to essentially the same average density; its icosahedral facets are flatter, and its shell is about 20% thinner, consistent with the smaller size of its major capsid protein. Otherwise, their major features are remarkably similar: CCV has the same triangulation number; its hexons and pentons also have chimney-like protrusions with an axial channel through each capsomer; and there are ''triplexes'' on the outer surface at the sites of local threefold symmetry. The basic herpesvirus capsid architecture is, therefore, remarkably well conserved in CCV and implies a utilitarian basis to this design. The protein composition of CCV mirrors that of HSV-1, except for the absence of the 12-kDa protein, VP26, which is dispensable for assembly in the HSV-1 system and, apparently, wholly dispensable for CCV.

INTRODUCTION

Herpesviruses form an extensive family of DNA-containing viruses that infect a wide range of vertebrate hosts. Over 100 members have been described and classified, on the basis of their biological properties, into three subfamilies: Alpha-, Beta-, and Gammaherpesvirinae. Their genomes are linear double-stranded molecules containing various arrangements of terminal or internal repeats and vary considerably in size, from ~125 kbp to ~230 kbp (Roizman, 1990). Despite their diversity of host range and genome size, herpesviruses are morphologically conservative and invariably exhibit a large (>100-nm-diameter) thick-walled nucleocapsid, surrounded by an envelope with an intervening proteinaceous layer (or "tegument"). Their appearance is distinctively different from that of any other animal virus. Key to this morphological conservatism is the capsid which, after assembling and undergoing DNA packaging in the nucleus, becomes wrapped with the tegument and envelope (review: Rixon, 1993). Thus the size and shape of the virion is imposed — at least, in part — by the capsid. The genes involved in capsid assembly are largely conserved — at least among mammalian herpesviruses, i.e., they are present and code for proteins of similar sizes, although not necessarily closely conserved in sequence (Davison, 1993).

Recently, progress has been made in defining the molecular anatomy of the capsid of the archetypal alphaherpesvirus, herpes simplex virus 1 (HSV-1). Its shell contains four major proteins (Table 1), whose assembly is controlled by interaction with two internal proteins coded by genes UL26 and UL26.5, respectively. The latter proteins are later proteolytically processed and (mostly) expelled, concomitant with DNA packaging (review: Steven and Spear, 1995). The capsid is icosahedrally symmetric, of triangulation class $T = 16$ (Wildy *et al.,* 1960). Its surface lattice is made up of 150 hexamers and 12 pentamers of VP5, with the other three shell proteins attached at specific sites on the outer surface. VP19c and VP23 form heterotrimeric complexes ("triplexes") at the sites of local threefold symmetry (Newcomb *et al.,* 1993), and six copies of VP26 are distributed around the outer tip of each hexon (Booy *et al.,* 1994).

Derivation of the foregoing account of the HSV-1 capsid depended heavily on the use of cryo-electron microscopy and three-dimensional image reconstruction (reviews: Adrian *et al.,* 1984; Booy, 1992; Chiu, 1993). Localization of the four shell proteins was contingent on being

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able to modulate the capsid structure in biochemically defined ways — by extraction of specific components (Newcomb and Brown, 1991; Newcomb *et al.,* 1993), by complementing depleted capsids with purified proteins (Booy *et al.,* 1994), or by decorating capsids with antibodies (Trus *et al.,* 1992). Hitherto, the only herpesvirus other than HSV-1 whose capsid structure has been analyzed in comparable detail is equine herpes virus 1 (Baker *et al.,* 1990), a relatively closely related alphaherpesvirus.

In the present paper, we have extended these studies to channel catfish virus (CCV) (Wolf and Darlington, 1971). This virus has the gross morphology of a herpesvirus, although no specific relationship to mainstream herpesviruses was evident from an analysis of the proteins predicted from its complete DNA sequence (Davison, 1992). The motivation for these experiments was twofold: (i) to complement the studies cited above by exploiting evolution as an alternative modulator of herpesvirus capsid structure; and (ii) in view of the large evolutionary distance between CCV and HSV-1, this comparison afforded an opportunity to identify those capsid features which have been most closely conserved, and which may therefore be regarded as functionally essential.

MATERIALS AND METHODS

Cultivation and isolation of capsids

CCV was grown in a fish cell line (BB) at 31° by infecting at a low multiplicity of infection (0.001 PFU/cell). Three days after infection, cells were homogenized and capsids were isolated by sucrose gradient centrifugation, as described by Davison and Davison (1995). The preparation studied here was the "U-band," which contains mainly empty capsids (equivalent to A-capsids of HSV-1) as well as a few that contain scaffolding proteins (equivalent to B-capsids of HSV-1). After dialysis to remove sucrose, the capsids were concentrated by centrifugation.

Cryoelectron microscopy

Purified capsids were prepared for cryoelectron microscopy and observed as described previously (Booy *et al.,* 1991). In brief, micrographs were recorded at a nominal magnification of 36,000×, using low-dose techniques, on a Philips EM400T equipped with a Gatan Model 626 cryoholder and modified Gatan anticontamination blades (Gatan, Warrendale, PA).

Image processing and three-dimensional reconstruction

The micrographs selected for reconstruction showed good distributions of particles, promising contrast, and had been recorded at defocus values such that the first zero of the phase-contrast transfer function was at $\sim(2.5\text{nm})^{-1}$ and identified as such by optical diffraction. Two such micrographs were digitized on a Perkin–Elmer 1010MG microdensitometer at a sampling rate of ~0.86nm per pixel. The dimensions of the CCV capsid were calibrated relative to the axial spacing of the tails of bacteriophage T4 particles mixed with the CCV capsids. This spacing, to which a value of 4.05 nm was assigned (Moody and Makowski, 1981), was measured to within 1% standard deviation from diffraction patterns. Reconstructions were calculated by "common lines" techniques of Fourier analysis (Crowther, 1971; Fuller, 1987; Baker *et al.,* 1988, 1989) as described previously (Trus *et al.,* 1992; Conway *et al.,* 1993) and making use of an Intel iPSC/860 massively parallel supercomputer (Johnson *et al.,* 1994). Particle orientations were also refined using the iterative procedure described by Cheng *et al.,* (1994).

The surface shells of HSV-1 A-capsids (empty), B-capsids (which contain scaffolding proteins but no DNA), and C-capsids (which contain DNA) are structurally indistinguishable at current resolutions. For convenience, we used the B-capsid reconstruction reported by Conway *et al.* (1995), to compare with CCV. Although these data were sufficient for a density map at 2.4-nm resolution (Conway *et al.,* 1995), the map used in the present comparison was restricted to 3-nm resolution to rule out any complications that might arise from differences in resolution. The HSV-1 capsid dimensions, calibrated as described above (B.L.T. and F.P.B., unpublished results), confirm previously reported data (Booy *et al.,* 1991; Newcomb *et al.,* 1993).

RESULTS AND DISCUSSION

A field of vitrified CCV capsids is shown in Fig. 1. Most are empty. In appearance, they generally resemble capsids of HSV-1 (Schrag *et al.,* 1989) or equine herpes virus 1 (Baker *et al.,* 1990), except that they are somewhat smaller and more angular. Three-dimensional reconstructions were calculated separately from two cryoelectron micrographs, combining 81 capsids and 78 capsids, respectively. They yielded consistent results. Subsequently, the 140 best particles were combined in a composite density map (Figs. 2a, 2c, 2e, and 3a), whose resolution extended to ~3.0 nm according to the criterion of Conway *et al.* (1993). The exterior and interior surfaces of the CCV capsid shell as viewed along an axis of twofold symmetry are shown in Figs. 2a and 2c, respectively. The $T = 16$ triangulation geometry is apparent. The hexons and pentons have prominent chimney-like protrusions that extend outward from the inner layer where intercapsomer contacts take place. There is an axial channel through each capsomer with a constriction near the middle, and there are triplexes on the outer surface at the sites of local three-fold symmetry (cf. Figs. 3a and 3b).

In Fig. 2, CCV is compared with HSV-1 at the same resolution. The facets of the CCV capsid are somewhat

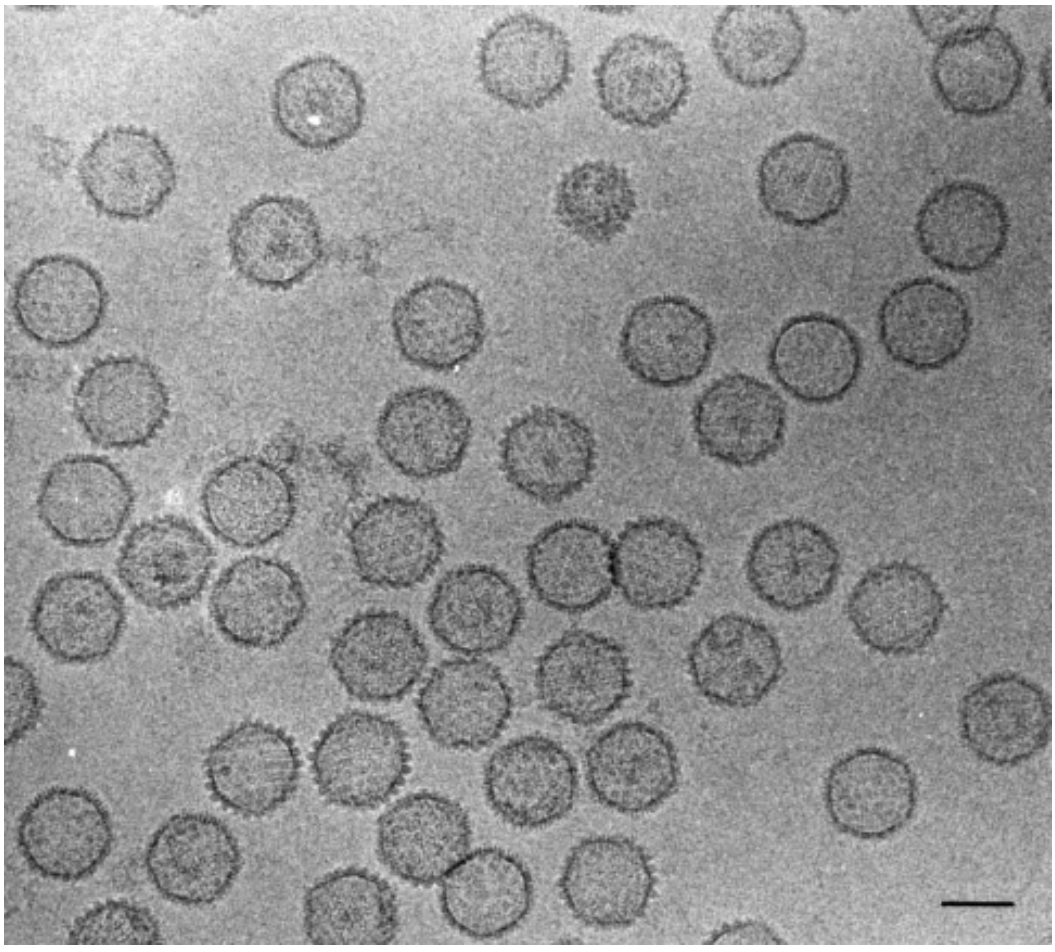

FIG. 1. Cryoelectron micrograph of purified capsids of channel catfish virus. This preparation contains predominantly empty capsids, which are equivalent to A-capsids of HSV-1. Bar, 100 nm.

flatter than those of HSV-1 and its shell is about 20% thinner (i.e., CCV hexons are 12.4 nm thick, compared to 15 nm for HSV-1; cf. Fig. 3c). The CCV capsid is significantly smaller, with an average outer diameter of 116.7 nm compared to 124.8 nm for HSV-1, as measured from spherically averaged radial density profiles (Fig. 3c). The experimental uncertainties are ~1%. Other dimensions of the CCV capsid are 128.3 nm vertex to vertex, i.e., penton tip to penton tip, and 106.8 nm between the centers of opposing facets, i.e., C-hexon[2] tip to C-hexon tip. The corresponding figures for HSV-1 are 135.3 and 120.9 nm, respectively. The ratio between these diameters (i.e., fivefold:threefold) is 1.20 for CCV compared with 1.12 for HSV-1, which provides a quantitative statement in relation to its flatter facets. Despite these differences in dimensions, the major morphological features of the CCV capsid are strongly reminiscent of those of HSV-1 (cf., Conway *et al.,* 1993; Zhou *et al.,* 1994) or EHV-1 (Baker *et al.,* 1990), and we conclude that the structural organization of alphaherpesvirus capsids is emulated remarkably well in the case of CCV.

It is noteworthy that the observed size differential between CCV and HSV-1 capsids correlates with the sizes of their respective genomes: CCV, 134 kbp (Davison 1992); HSV-1, 152.3 kbp (McGeoch *et al.,* 1988). Their average internal diameters, as measured from the respective radial density profiles (Fig. 3c), are 91.9 and 94.8 nm and correspond to a volume ratio of 0.91. The ratio between the masses of their respective genomes is 0.88. It follows that average density of encapsidated DNA must be very similar in both viruses, there being no evidence for the retention of internal proteins in the fully packaged capsids in amounts that are likely to affect this conclusion (Booy *et al.,* 1991; Davison and Davison, 1995). To extrapolate from this observation, herpesviruses with larger genomes are likely to have commensurately larger capsids. For instance, one would expect the capsid of human cytomegalovirus (229 kbp; Bankier *et al.,* 1991) to be significantly larger than that of HSV-1, unless a higher density of packaged DNA is achieved in this system. It would contribute to

[2] C-hexons, E-hexons, and P-hexons are distinguished according to their sites on the icosahedral surface lattice — Steven *et al.* (1986).

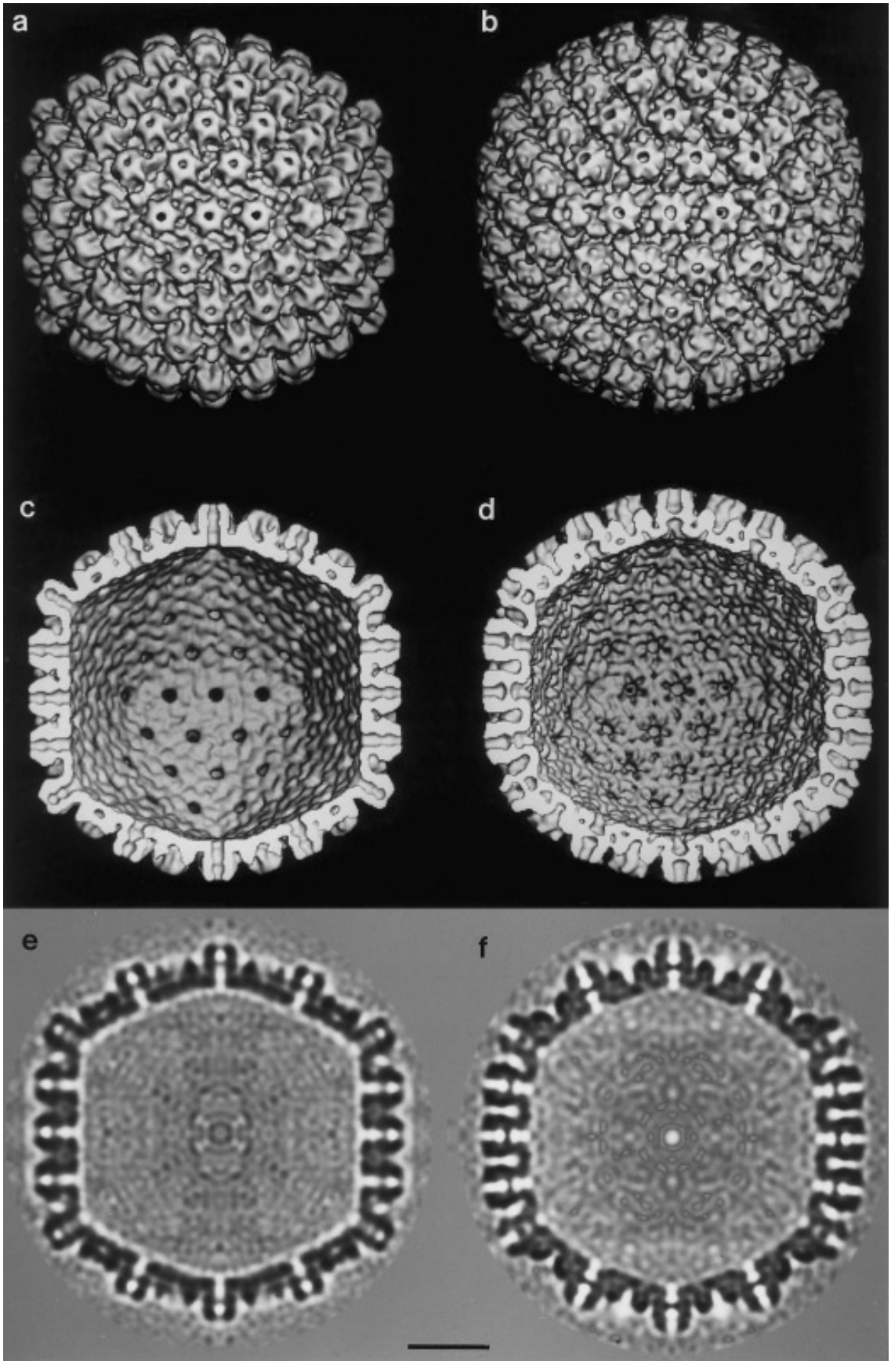

FIG. 2. Comparison of the three-dimensional capsid structures of CCV (a, c, e) and HSV-1 (b, d, f). The capsids are viewed along a twofold symmetry axis. Outer surface, (a) and (b); inner surfaces, (c) and (d); central thin sections, (e) and (f). The distribution of capsomers (a and b) is distinctive for the triangulation number, $T = 16$, with lines of three hexons connecting pentons along each edge of the icosahedral surface lattice. Bar, 25 nm.

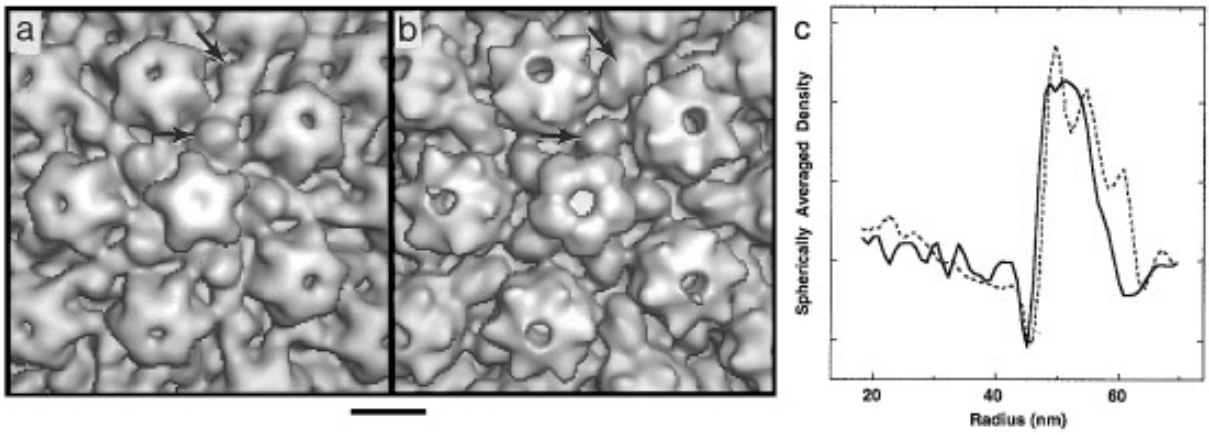

FIG. 3. Three-dimensional reconstructions at 3.0-nm resolution of the capsids of channel catfish virus (a) and herpes simplex virus 1 (b) as viewed along a fivefold axis of symmetry. Shown in each panel are blow-ups of the capsid structure centered on a penton, surrounded by five P-hexons (i.e., peripentonal hexons). The triplexes occupy each site of local threefold symmetry, i.e., sites surrounded by three capsomers (pentons or hexons); two triplexes, indicative of the sets surrounded by two P-hexons and a penton and by two P-hexons and one C-hexon, respectively, are indicated by arrows in (a) and (b). Bar, 10 nm. (c) Radial density profiles (plotted in arbitrary units) of empty capsids of CCV (solid curve) and HSV-1 (dotted curve) were obtained by spherically averaging the respective density maps.

a more general understanding of herpesvirus capsid morphogenesis to know whether such size differences occur, and if so, whether they reflect (as we would suspect) different-size capsomers, i.e., with larger diameters relative to their central axes, rather than a change in *T* number.

Protein composition of CCV

Recently, the capsid proteins of CCV have been identified as the products of specific genes by application of high-resolution mass spectrometric techniques, and their copy numbers determined by quantitative SDS–PAGE (Davison and Davison, 1995). To facilitate comparison, these data are reproduced in Table 1.

VP26 of HSV-1 has no counterpart in CCV

VP26, at 12 kDa, is the smallest capsid protein of HSV-1. It may be extracted from the capsid under relatively mild conditions that leave the shell otherwise intact (Newcomb and Brown, 1991; Newcomb *et al.,* 1993), indicating that VP26 does not play a significant role in capsid stabilization. Moreover, experiments with recombinant baculoviruses have indicated that VP26 is not required for capsid assembly (Thomsen *et al.,* 1994; Tatman *et al.,* 1994). Compositional analysis of purified CCV virions revealed no evidence for a protein in the same molecular weight range (Davison and Davison, 1995). Six copies of VP26 are distributed around the tip of each hexon on HSV-1 capsids (Booy *et al.,* 1994), where they form six little horn-like excrescences when visualized at resolutions of 3.0 nm or better (Zhou *et al.,* 1994; Conway *et al.,* 1993; Fig. 3b). No comparable features are seen on the CCV reconstruction (Fig. 3a), consistent with the absence of VP26-like subunits. We conclude that if CCV does have a counterpart to VP26, it must associated less securely with the capsid and have been lost from our capsid preparations.

TABLE 1

Capsid Shell Proteins of HSV-1 and CCV

HSV-1[a]				CCV[b]	
Protein	Gene	Copy no.	Site	Protein of gene	Copy no.
VP5	UL19	960	Hexons and pentons	39	960
VP19c	UL38	350	Triplexes	53	310
VP23	UL18	570	Triplexes	27	630
VP26	UL35	900[c]	Hexon tips	—	—

Note. Copy numbers of the triplex proteins were determined in proportion to the major capsid proteins (VP5 or gene 39 protein, respectively) by quantitative SDS–PAGE.

[a] Data from Newcomb *et al.* (1993).

[b] Data from Davison and Davison (1995).

[c] Data from Trus *et al.* (1995); Zhou *et al.* (1995).

Major capsid proteins

We assume that the gene 39 protein forms both the hexons and pentons of the CCV capsid, as VP5 does for HSV-1 (Newcomb *et al.,* 1993). At 123 kDa, this protein is ~21% smaller than VP5 (149 kDa), although it is still unusually large for a viral capsid protein. As noted above, the CCV capsid shell is ~20% thinner than that of the HSV-1 capsid, but the diameter of the CCV capsomers in its surface lattice is only about 3% smaller than those of HSV-1. Thus the main differences between them — apart from the absence of VP26 from the tips of the CCV hexons — are that its protrusions are 2.0–2.5 nm shorter, even when compared with HSV-1 capsomers that are missing VP26 (data not shown; cf. Booy *et al.,* 1994; Trus *et al.,* 1995). Otherwise, the predominant structural features, including the closely knit inner "floor" layer, and the constriction midway through the axial channel that runs through each capsomer, are common to both capsids (Figs. 2 and 3). Such differences as exist occur at the level of relatively minor details (cf. Figs. 3a and 3b).

There is insufficient homology between the gene 39 protein and VP5 to allow a convincing alignment of their respective sequences (Davison, 1992). Nevertheless, the structural resemblance between the respective capsomers suggests that the domainal organizations of the two proteins may be quite similar. In this context, the lower molecular weight of gene 39 protein implies that it may lack a domain (or domains) that is present in VP5. This putative domain would probably be located in the HSV-1 capsomer protrusions since their length is the most pronounced structural difference between the two capsids.

Triplex proteins

For HSV-1, the estimated copy numbers of VP19c and VP23 are both close to being integral multiples of the number of triplexes (320) (Newcomb *et al.,* 1989). These proteins have been identified as forming the triplexes on the basis of the observation that treatment of purified capsids with 2.0 *M* guanidine hydrochloride removed the two sets of triplexes (120 in all) closest to the pentons, concomitant with extraction of comparable fractions of both proteins (Newcomb *et al.,* 1993). We have not been able to obtain CCV capsids in sufficient quantities for similar experiments; nevertheless, we tentatively assign the CCV proteins coded by genes 53 and 27 as triplex components on the basis that the CCV capsid definitely possesses triplexes (Figs. 2a, 2c, 2e, and 3a), and the copy numbers of these proteins match those of VP19c and VP23, respectively. We also note that the molecular weight of CCV gene 27 protein is close to that of VP23 (Table 1). The significance of this match is enhanced by the consideration that inferred counterparts to VP23 are remarkably uniform in size (33–35 kDa) throughout the three mammalian herpesvirus subfamilies (Table 2). In contrast, the CCV gene 53 protein is substantially smaller than VP19c, although this protein is more variable in size among herpesviruses (Table 2). Again, no homology is evident between these CCV proteins and their putative HSV-1 counterparts (Davison, 1992).

TABLE 2

Molecular Weights of Major Capsid Proteins and Putative Triplex Proteins of Various Herpesviruses

Virus	Protein counterpart of VP5	Protein counterpart of VP19c	Protein counterpart of VP23
Alphaherpesvirinae			
Herpes simplex virus 1	149,082	50,263	34,270
Varicella zoster virus	154,978	53,971	34,309
Equine herpes virus 1	152,182	51,306	33,841
Betaherpesvirinae			
Human cytomegalovirus	153,871	33,027	34,954
Human herpesvirus 6[a]	151,949	34,205	33,464
Gammaherpesvirinae			
Epstein–Barr virus	153,916	39,190	33,624
Herpesvirus saimiri	154,354	37,385	34,256
Equine herpes virus 2[b]	153,202	37,509	33,418
Unclassified			
Channel catfish virus	123,008	34,677	31,781

Note. These proteins were identified as counterparts of VP19c and VP23 of HSV-1 on the basis of homologies observed among their amino acid sequences (as translated from the corresponding gene sequences using the Peptidesort routine of the GCG program), and on the relative positioning of these genes in the respective genomes (cf. Davison, 1993).

[a] From Gompels *et al.* (1995).

[b] EHV-2 was originally thought to be a betaherpesvirus but is now characterized as a gammaherpesvirus on the basis of its genome sequence (Telford *et al.,* 1995).

Essential structural elements — Capsomer protrusions and triplexes

The presence and seemingly conserved subunit composition of triplexes implies that they are an important feature of herpesvirus capsid design. Evidence, both from null mutants in HSV-1 genes UL18 (coding for VP23) (Desai *et al.,* 1993) and UL38 (coding for VP19c) (Pertuiset *et al.,* 1989) and from recombinant baculovirus expression of HSV-1 capsid proteins (Thomsen *et al.,* 1994; Tatman *et al.,* 1994), indicates that both triplex proteins are required for capsid morphogenesis, although their precise roles have yet to be defined. In addition, it has been proposed that they serve to stabilize the mature capsid by reinforcing the bonding between neighboring capsomers (Baker *et al.,* 1990).

The capsomer protrusions rise 8–9 nm above the CCV capsid base, and ~11 nm in the case of HSV-1. The fact that these protrusions — which represent a major

biosynthetic investment — are common to CCV and HSV-1 attributes functional significance to them. As to what function(s) might be involved, we can as yet only speculate. Since there is no direct contact between neighboring protrusions, they are clearly not required for intercapsomer interactions. However, they may participate in intracapsomer stabilization; they may provide conduits for the exit of scaffolding proteins or the passage of DNA (Baker *et al.,* 1990; Booy *et al.,* 1991), and they presumably contain sites for linking the capsid to the tegument. However, it is not obvious why such elaborate structures should be required for any of these purposes.

Evolutionary implications

The observed conservation of overall architecture, principal structural features, and molecular composition (apart from VP26) between the capsids of CCV and HSV-1 attests to a remarkably stable and successful molecular design. This conservatism at the level of large-scale three-dimensional structure stands in marked contrast to the absence of discernible amino acid sequence homology between corresponding capsid proteins. This paradox is reminiscent of observations that some proteins with unrecognizably different sequences fold into essentially the same conformations (e.g., Orengo *et al.,* 1993). The much greater size and complexity of herpesvirus capsids point to an even more remarkable instance of the same general phenomenon. In this context, an intriguing issue is whether corresponding proteins in CCV and HSV-1 have homologous folds, or whether it is only the three-dimensional design of the full icosahedral shell that is invariant.

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