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Authors

Baker, TS
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Booy, FP
et al.

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THREE-DIMENSIONAL RECONSTRUCTIONS OF 'LIGHT' AND 'INTERMEDIATE' CAPSIDS OF EQUINE HERPES VIRUS

T. S. Baker,* W. W. Newcomb,† F. P. Booy,‡ J. C. Brown,† and A. C. Steven‡

***Department of Biological Sciences, Purdue University, West Lafayette, IN 47907**
†Department of Microbiology, University of Virginia Medical School, Charlottesville, VA 22908
‡Laboratory of Physical Biology, NIAMS/NIH, Bethesda, MD 20892

Equine herpes virus type 1 (EHV-1) belongs to an extensive family of large, genetically complex, and medically important animal viruses. The virion consists of an icosahedral nucleocapsid (T=16) separated from the viral envelope by a proteinaceous tegument layer. Assembly occurs in the nucleus of infected cells where capsids assemble, are packaged with DNA, then bud through the nuclear membrane. Two morphological species of EHV-1 capsids have been distinguished:[1] "lights" which are abortive particles incapable of packaging DNA and "intermediates" which are precursors in the assembly of mature virions.[2] Purified "intermediates" contain an additional protein, VP22 (46 kDa), which is not present in "lights" and accounts for ~10% of the total particle mass.[3] In order to characterize the capsid structures of the two particle types and explore differences between them, we have applied three-dimensional reconstruction techniques to electron micrographs of frozen-hydrated specimens.[4-6]

The Kentucky A strain of EHV-1 was propagated in L-929 cells, and the two types of capsids were obtained as separate fractions from Renografin-76 density gradients. Neither fraction contained any significant amount of DNA. Cryo-electron microscopy of capsids on carbon film substrates was performed as described earlier.[7] Micrographs were recorded at a nominal magnification of ×36,000 at 2-3μm underfocus and digitized with a 50μm step size (~1.38nm sampling at the specimen). Three-dimensional reconstructions of the two particle types were computed to 4.5nm resolution using modified "common-lines" procedures.[4-6] Images of 37 "light" and 39 "intermediate" particles were separately combined to compute three-dimensional density distributions.

Cryo-electron micrographs of "lights" (Fig. 1a) and "intermediates" (Fig. 1b) show particles whose external morphologies and dimensions are quite similar. The "intermediates" appear to have additional mass, non-uniformly distributed inside the capsid shell. The surface structures revealed in the two reconstructions are closely similar (Fig. 2). Both surfaces consist of 162 prominent, protruding capsomers: the twelve at the icosahedral vertices (pentavalent positions) are pentameric in shape and the remaining 150, at the hexavalent positions, are hexameric in shape which is consistent with previous results.[8] The center of each capsomer contains a hollow channel which appears to extend completely through the capsid wall. This feature is revealed in sections through the center of each reconstruction (Fig. 3) where the different types of capsomer are seen in cross-section. The pentavalent capsomers extend to a larger radius (~64 nm) than any of the hexavalent capsomers. The thickest dimension in both capsid shells (~14.2 nm) is from the inner wall of the capsid to the outermost tip of the hexavalent capsomers. The thinnest region of the capsid wall (~5 nm) is from the inner surface to the "pits" located between adjacent pairs of capsomers. The inner surface of both capsids is relatively smooth and featureless except for the openings leading to the central channels of the capsomers. At the 320 local three-fold positions between capsomers on the outer surface, there are well defined accumulations of density (cf. Fig. 3). The openings beneath the hexavalent capsomers are larger (~4nm across) than those beneath the pentavalent capsomers.

The only striking difference between the structures of the "lights" and "intermediates" is the markedly higher level of background density inside the "intermediates" (Fig. 3a,b). This presumably arises when the non-uniformly distributed mass observed in the unprocessed images (Fig. 1b) is averaged. We conclude that, in "intermediates", the extra mass located inside the capsid most likely represents the presence of VP22 which is not uniformly or symmetrically arranged.[9]

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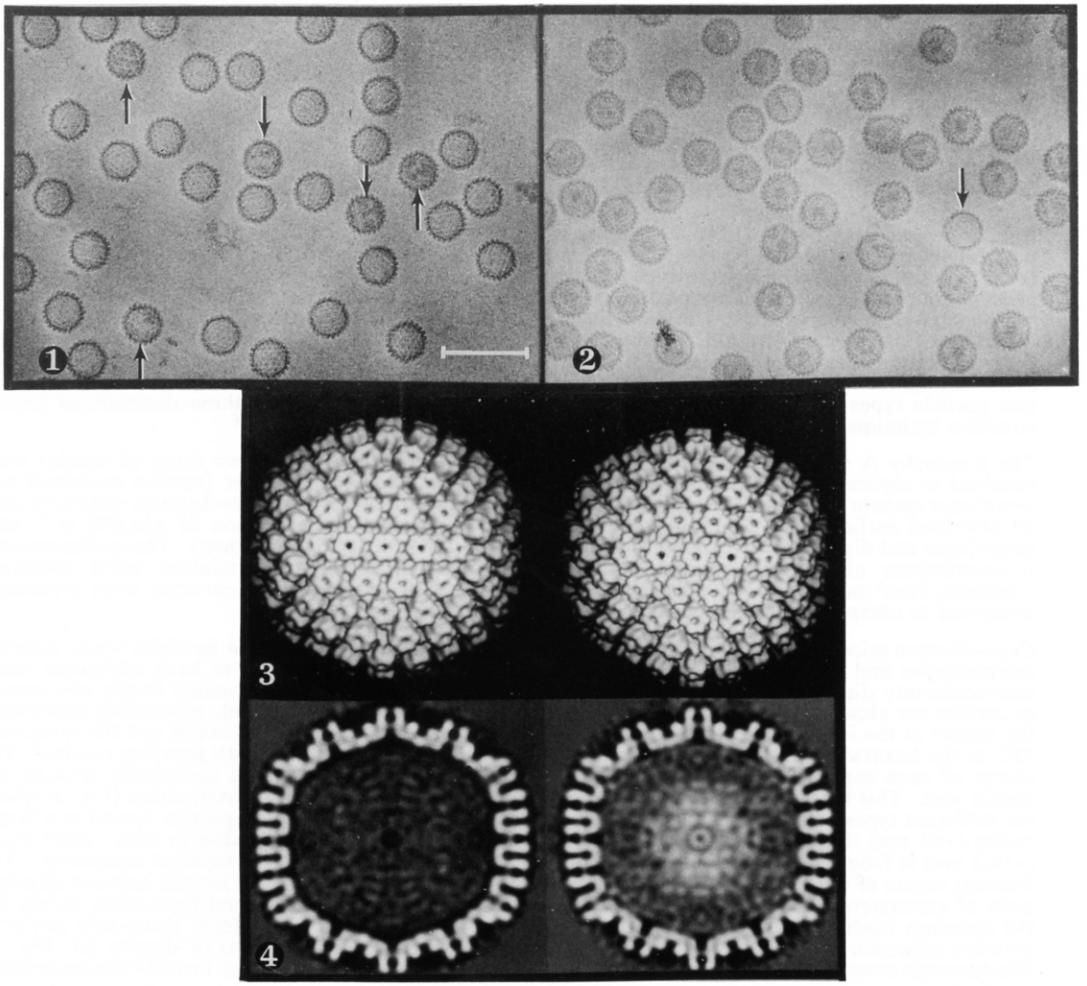

FIG. 1. -- Micrograph of frozen-hydrated EHV-1 "light" sample. A few "intermediates" are also present (arrows). Microscope defocus level ≃2.8μm underfocus. Bar = 250 nm.

FIG. 2. -- Micrograph of frozen-hydrated EHV-1 "intermediate" sample. A single empty capsid is identified by the arrow. Additional density, not observed in "lights", appears to be non-uniformly distributed, but localized primarily near the capsid centers. Microscope defocus level ≃2.2μm underfocus. Magnification same as Fig. 1.

FIG. 3. -- Surface shaded views of the three-dimensional reconstructions of "light" (left) and "intermediate" (right) EHV-1 capsids, viewed along icosahedral 2-fold symmetry axes. The main features of both surfaces are nearly identical indicating that the extra protein (VP22) present in "intermediates" is not part of the surface structure.

FIG. 4. -- Cross-sections through the centers of the "light" (left) and "intermediate" (right) reconstructions in the 2-fold view. The highest densities (brightest regions) in both reconstructions appear to have nearly identical distributions indicating that the two capsids have the same structure. The presence of VP22 in the "intermediates" correlates with the higher average density level in the center compared to "lights".