Primary Envelopment of the Herpes Simplex 1 Virion

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Herpes simplex virus type 1 (HSV-1), a typical herpesvirus, initially assembles in the nucleus of the infected cell, producing a procapsid, 125 nm in diameter, conforming to icosahedral symmetry. The procapsid is then packaged with the DNA genome and undergoes a major structural transformation [1]. This particle, the nucleocapsid or C-capsid, migrates out of the nucleus. To do so, it first buds through the inner nuclear membrane and becomes coated with a layer of the NEC (nuclear egress complex) protein. This yields a Primary Enveloped Virion (PEV) [2]. Its envelope then fuses with the outer nuclear membrane, releasing the nucleocapsid into the cytoplasm. Subsequent steps in the pathway include acquisition of the viral envelope (a membrane populated with glycoprotein spikes) and the tegument (a protein compartment between the envelope and the capsid).

The PEV is key to nuclear egress but has been difficult to study because of its transient nature. We used a mutant [3] that causes PEVs to accumulate in the perinuclear space between the inner and outer nuclear membranes. These PEVs were harvested by isolating infected cell nuclei, disrupting them, and fractionating the lysates by differential centrifugation. These preparations were imaged by cryo-electron tomography and cryo-electron microscopy with three-dimensional reconstruction.

In Fig. 1, we compare central slices from cryo-tomograms of a PEV containing a C-capsid (A) and one whose capsid is almost empty (B). In both, the capsid is surrounded by an 8.5 nm-thick spherical shell and an envelope. Panel C is from a mature enveloped virion (MEV) - the end-product of the assembly pathway. The MEV is ~ 20% larger than the PEV and its outer surface is densely packed with glycoprotein spikes; in contrast, PEVs have very few spikes. The greater size of the MEV is due to its tegument which is much more capacious than the NEC layer of a PEV and to its spikes. It follows that tegument proteins are acquired at a later stage of the pathway, as PEVs have little space to accommodate them. The differing complements of spike proteins for PEVs and MEVs imply that membrane fusion in primary envelopment is fundamentally different from the fusion event whereby the MEV enters a host cell. In Fig. 2, we compare cryo-EM reconstructions of (A) the PEV; (D) the C-capsid; and (G) a B-capsid that contains no DNA but retains the protein scaffold of the procapsid. Below are blow-ups of regions that differ in their occupancies of two sites. The heterodimer UL17/UL25 is present at sites around the capsid vertices in the PEV and the C-capsid but not in the B-capsid (white arrows in C, F and I). The VP26 protein is present on the PEV and the C-capsid (arrowheads in panels B and E) but not on this B-capsid, which is known to lack VP26 (data not shown). These and other observations suggest that it is a network of NEC - UL17/UL25 interactions that drives budding of the PEV.

References:
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Figure 1. Near-central slices through cryo-electron tomograms of (A and B) Primary Enveloped Virions and (C) a Mature Enveloped Virion. All three particles contain an icosahedral capsid that is fully filled with DNA in (A) and (C) and contains low-density material, probably under-packaged DNA, in (B). Scale bar (C) = 50 nm.

Figure 2. Central sections from 3D reconstructions of (A) the PEV; (D) the C-capsid; (G) a B-capsid produced by in vitro maturation of procapsids. The resolution is ~ 2 nm. The capsids are viewed along an axis of 2-fold symmetry. The panels in the lower row compare the respective occupancies of two viral proteins. The arrowheads in (B) and (E) point to spurs of density representing VP26 subunits bound to the outer tips of hexons of the major capsid protein. The arrows in (C), (F) and (I) point to sites where UL17/UL25 dimers bind around the 5-fold vertices. This density has ~100% occupancy in (C), ~ 60% in (F), and ~ 0% in (I). The white contours delineate the “triplex” proteins that bind UL17/UL25. Scale bars = 50 nm (G) and 10 nm (I).