The Significance of the Golgi complex in Herpes Virus Morphogenesis

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Herpes Viruses comprise capsid, tegument and envelope with embedded glycoproteins. Capsids assemble in the host cell nucleus and are translocated into the perinuclear space (PNS) by budding at the inner nuclear membrane (INM) acquiring tegument and envelope. It is assumed that virions escape the PNS by fusion of the viral envelope with the outer nuclear membrane (ONM) releasing capsid and tegument into the cytoplasmic matrix [1]. Infectious progeny virus is believed to originate exclusively by budding of these cytoplasmic capsids at membranes of the trans Golgi network. Interestingly, the Golgi complex fragments as early as 15 h post infection (hpi) [2] questioning the essential role of the Golgi complex in herpes virus envelopment. We therefore investigated the function of the Golgi complex in the replicative cycle of herpes simplex virus 1 (HSV-1) and bovine herpes virus 1 (BHV-1), two members of the α-herpes virus family, by electron microscopy employing protocols that enable imaging at high spatial and high temporal resolution [3]. We found that both HSV-1 and BHV-1 bud at membranes at any site of intact Golgi complexes early in infection. In addition, Golgi cisternae were filled with virions and showed clear indications of fission, suggesting that virions followed another pathway than by budding, e.g. by intraluminal transportation from the PNS [4]. Connectivity between the ONM, RER and Golgi complex [5] support the idea of intraluminal transportation. After fragmentation, which is probably the result of vacuole formation, intraluminal viral transportation is probably impeded leading to accumulation of virions in the PNS-RER compartment. Exposure of infected cells to Brefeldin A (BFA) leads to immediate disassembly of the Golgi complex [6] mimicking Golgi fragmentation. The result is accumulation of virions in the PNS-RER compartment. Interestingly, these virions are infective, indicating that capsids acquire all essential proteins during budding at the INM.

To demonstrate that virions of the PNS are infective, we investigated a HSV-1 mutant lacking US3 (R7041ΔUS3; gift from B. Roizman, University of Chicago) which is involved in regulation of the translocation of capsids from the necleus to the cytoplasm. 98% of virions derived by budding at the INM get stuck in the PNS. The Golgi complex is not involved in envelopment at all. Yields of infectious progeny virus equal that of wild type (wt) HSV-1 clearly indicating that R7041ΔUS3 virions derived by budding at the INM are infective. Since US3 is not a virion component composition of R7041ΔUS3 and wt HSV-1 derived by budding at the INM is identical, and hence, wt HSV-1 of the PNS can be assumed to be infective.

The data clearly show that the Golgi complex is not essential for envelopment per se. Its main function is to provide functional proteins, e.g. glycolysation. These proteins need to be transported into the nucleus or to nuclear membranes. As shown for glycoprotein D,

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detectable insertion into nuclear membranes starts between 5 and 6 hpi. Budding at the INM starts about 6 hpi, maximal budding activity is about 12 hpi [7]. Virus yields at 20 hpi after exposure to BFA prior to 5 hpi is zero. Virus yield after exposure to BFA at 8 hpi is substantially increased indicating that proteins transported to the nucleus are fully active.

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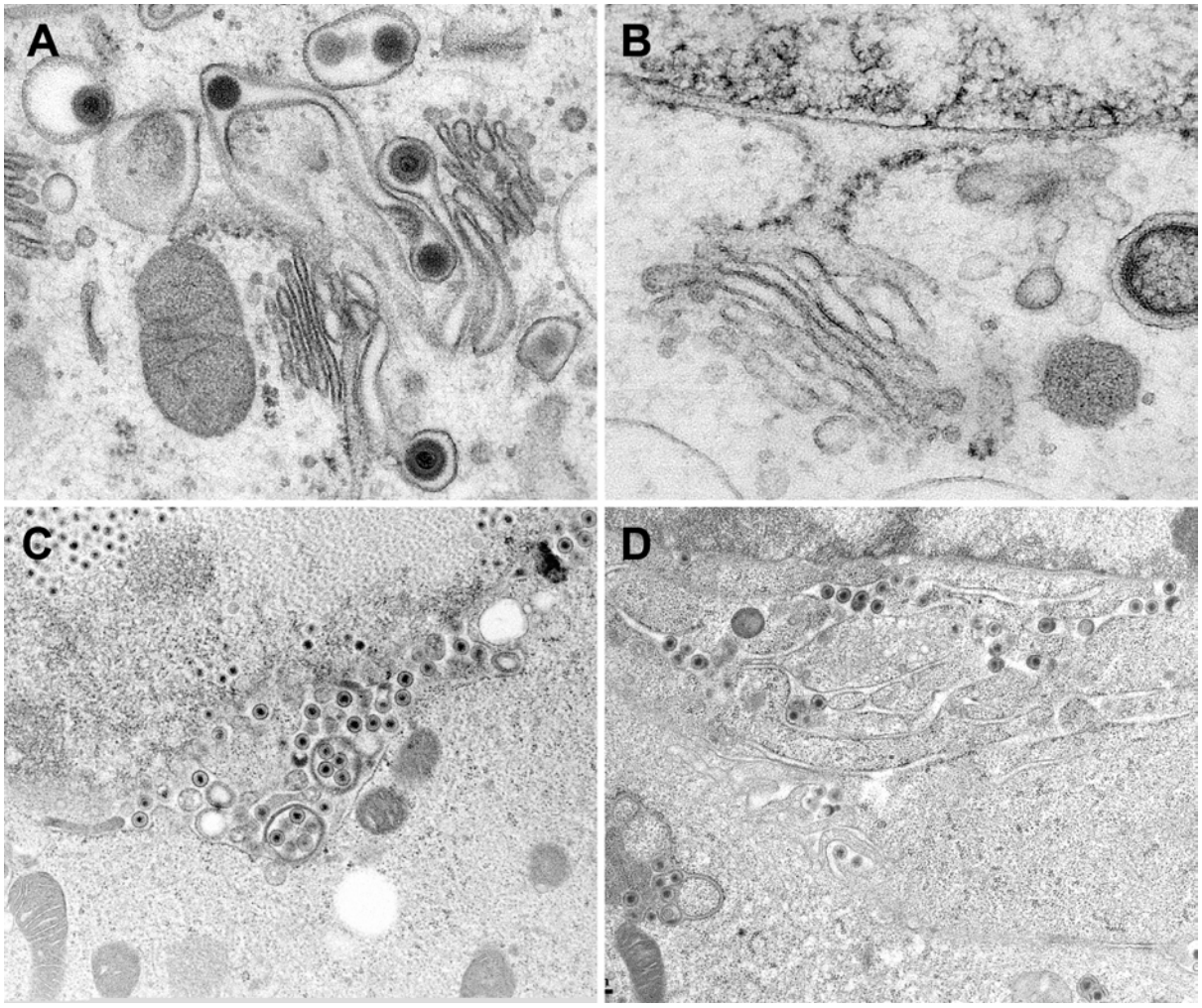

Figure 1. Images of herpes virus infected Vero cells. A) Golgi cisternae filled with BHV-1 showing indications for fission. B) Connectivity between Golgi membranes and ONM. C) Accumulation of infectious HSV-1 virions lacking US3 in the PNS. D) Accumulation of virions at 20 hpi within RER after BFA treatment at 16 hpi.

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